

**TOCOPHEROLS, TOCOTRIENOLS, OTHER CHROMAN AND SIDE
CHAIN DERIVATIVES AND USES THEREOF**

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BACKGROUND OF THE INVENTION

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Cross-Reference to Related Applications

This application is a divisional of pending U.S. Serial No. 09/502,592, filed February 11, 2000, which is a continuation-in-part of U.S. Serial No. 09/404,001, filed September 23, 1999, issued as U.S. 15 6,417,223 on July 9, 2001, which claims benefit of provisional application U.S. Serial No. 60/101,542, filed September 23, 1998, now abandoned.

Field of the Invention

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The present invention relates generally to the fields of organic chemistry and antiproliferative and pro-apoptotic compounds.

More specifically, the present invention relates to chroman-based compounds and derivatives and analogs thereof, and their uses as cell anti-proliferative, proapoptotic, immunomodulating, and anti-viral agents.

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Description of the Related Art

The biology of cell proliferation and cell death (apoptosis) is extremely complex, involving multiple intracellular signaling pathways and multiple interacting gene products. Cancer cells may exhibit multiple defects in normal regulatory controls of cell proliferation which allow them to increase in number. Furthermore, cancer cells exhibit defects in mechanisms that are involved in eliminating abnormal cells by multi-step processes referred to as programmed cell death or apoptosis. Thus, combinations of unregulated cell proliferation and suppression of death inducing signaling pathways give cancer cells both growth and survival advantages.

Whether a cell increases in numbers or not depends on a balance of expression of negatively-acting and positively-acting growth regulatory gene products, and the presence or absence of functional cell death signaling pathways. Negative-acting growth regulatory genes

contribute to blockage of cells in the cell cycle. Positive-acting growth regulatory genes stimulate cells to progress through the cell cycle. Genes involved in apoptosis can be either proapoptotic or antiapoptotic, and the dynamic balance between them determines
5 whether a cell lives or dies.

Cancer cells, in order to survive and increase their numbers, undergo a series of mutational events over time that remove regulatory controls that give them the ability to grow unchecked and survive even in the presence of proapoptotic signals, and develop
10 attributes that permit them to escape detection and removal by the immune response defense system. Cancers may cause death of individuals unless removed by surgery or effectively treated with drugs.

A wide variety of pathological cell proliferative conditions exist for which novel therapeutic strategies and agents are needed to
15 provide therapeutic benefits. These pathological conditions may occur in almost all cell types capable of abnormal cell proliferation or abnormal responsiveness to cell death signals. Among the cell types that exhibit pathological or abnormal growth and death characteristics are (1) fibroblasts, (2) vascular endothelial cells, and (3) epithelial
20 cells. Thus, novel methods are needed to treat local or disseminated

pathological conditions in all or almost all organ and tissue systems of individuals.

Most cancers, whether they be male specific such as prostate or testicular, or female specific such as breast, ovarian or
5 cervical or whether they affect males and females equally such as liver, skin or lung, with time undergo increased genetic lesions and epigenetic events, and eventually become highly metastatic and difficult to treat. Surgical removal of localized cancers has proven effective only when the cancer has not spread beyond the primary
10 lesion. Once the cancer has spread to other tissues and organs, the surgical procedures must be supplemented with other more specific procedures to eradicate the diseased or malignant cells. Most of the commonly utilized supplementary procedures for treating diseased or malignant cells such as chemotherapy or bioradiation are not localized
15 to the tumor cells and, although they have a proportionally greater destructive effect on malignant cells, often affect normal cells to some extent.

Some derivatives of tocopherols, tocotrienols and vitamin E have been used as proapoptotic and DNA synthesis inhibiting agents.
20 Structurally, vitamin E is composed of a chromanol head and an alkyl side chain. There are eight major naturally occurring forms of vitamin

E: alpha (α), beta (β), gamma (γ), and delta (δ) tocopherols and α , β , γ , and δ tocotrienols. Tocopherols differ from tocotrienols in that they have a saturated phytyl side chain rather than an unsaturated isoprenyl side chain. The four forms of tocopherols and tocotrienols differ in the number of methyl groups on the chromanol head (α has three, β and γ have two and δ has one).

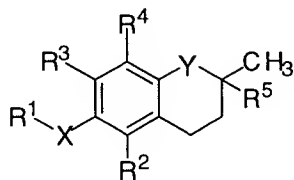
RRR- α -tocopheryl succinate is a derivative of RRR- α -tocopherol that has been structurally modified via an ester linkage to contain a succinyl moiety instead of a hydroxyl moiety at the 6-position of the chroman head. This ester linked succinate moiety of RRR- α -tocopherol has been the most potent form of vitamin E affecting the biological actions of triggering apoptosis and inhibiting DNA synthesis. This form of vitamin E induces tumor cells to undergo apoptosis, while having no apoptotic inducing effects on normal cells. The major advantage of this form of vitamin E as an anticancer agent is that many cancer cells either express low levels of esterases or do not express esterases that can cleave the succinate moiety, thereby converting the succinate form of RRR- α -tocopherol to the free RRR- α -tocopherol. RRR- α -tocopherol exhibits neither potent antiproliferative nor apoptotic triggering biological activity. However, the ester-linked vitamin E succinate is ineffective in vivo since natural esterases in the

host cleave off the succinate moiety, rendering an ineffective anticancer agent, RRR- α -tocopherol.

The prior art is deficient in the lack of effective means of inhibiting undesirable or uncontrollable cell proliferation in a wide variety of pathophysiological conditions while having no to little effect on normal cells. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

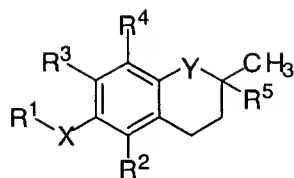
In one embodiment of the present invention, there is provided a compound having a structural formula



wherein X is oxygen, nitrogen or sulfur; Y is selected from the group consisting of oxygen, nitrogen and sulfur wherein when Y is oxygen or nitrogen, n is 1 and when Y is sulfur, n is 0; R¹ is selected from the

group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, carboxylic acid, carboxylate, carboxamide, ester, thioamide, thiolester, thiolacid, saccharide, alkoxy-linked saccharide, amine, sulfonate, sulfate, phosphate, alcohol, ether and nitrile; R^2 is selected from the group
5 consisting of hydrogen, methyl, benzyl carboxylic acid, benzyl carboxylate, benzyl carboxamide, benzyl ester, saccharide and amine; R^3 is selected from the group consisting of hydrogen, methyl, benzyl carboxylic acid, benzyl carboxylate, benzyl carboxamide, benzylester, saccharide and amine; R^4 is selected from the group consisting of
10 methyl, benzyl carboxylic acid, benzyl carboxylate, benzyl carboxamide, benzylester, saccharide and amine; and R^5 is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, carboxyl, amide and ester; wherein when Y is nitrogen, said nitrogen is substituted with R^6 , wherein R^6 is selected from the group consisting of
15 hydrogen and methyl; wherein when X is oxygen, R^2 is methyl, R^3 is methyl, R^4 is methyl and R^5 is phytyl, R^1 is not butyric acid.

In another embodiment of the present invention, there is provided a method for the treatment of a cell proliferative disease comprising administering to an animal a pharmacologically effective
20 dose of a compound having a structural formula



wherein X is oxygen, nitrogen or sulfur; Y is selected from the group consisting of oxygen, nitrogen and sulfur wherein when Y is oxygen or nitrogen, n is 1 and when Y is sulfur, n is 0; R¹ is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, carboxylic acid, carboxylate, carboxamide, ester, thioamide, thiolester, thiolacid, saccharide, alkoxy-linked saccharide, amine, sulfonate, sulfate, phosphate, alcohol, ether and nitrile; R² is selected from the group consisting of hydrogen, methyl, benzyl carboxylic acid, benzyl carboxylate, benzyl carboxamide, benzyl ester, saccharide and amine; R³ is selected from the group consisting of hydrogen, methyl, benzyl carboxylic acid, benzyl carboxylate, benzyl carboxamide, benzylester, saccharide and amine; R⁴ is selected from the group consisting of methyl, benzyl carboxylic acid, benzyl carboxylate, benzyl carboxamide, benzylester, saccharide and amine; and R⁵ is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl, carboxyl, amide and ester; wherein when Y is nitrogen, said nitrogen is substituted with R⁶, wherein R⁶ is selected from the group consisting of hydrogen and methyl..

In yet another embodiment of the present invention, there is provided a pharmaceutical composition comprising a compound disclosed herein and a pharmaceutically acceptable carrier.

5 In yet another embodiment of the present invention, there is provided a method of inducing apoptosis of a cell, comprising the step of contacting said cell with a pharmacologically effective dose of a compound of the present invention.

Other and further aspects, features, benefits, and advantages of the present invention will be apparent from the following
10 description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

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So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention are briefly summarized. above
20 may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of

the specification. It is to be noted; however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1 shows general structure of tocopherol, 5 tocotrienol and other chroman-based compounds.

Figure 2 shows general tocopherol-based compounds **1-29** presently synthesized and tested.

Figures 3A, 3B and 3C shows general synthetic organic approaches for the chemical variation of chromanol compounds at 10 position R^1 .

Figure 4 shows general synthetic organic approaches for the chemical variation of chromanol compounds at position R^2 .

Figure 5 shows general synthetic organic approaches for the chemical variation of chromanol compounds at position R^3 and R^4 .

15 **Figure 6** shows general synthetic organic approaches for the chemical variation of chromanol compounds at position R^5 .

Figure 7 shows general synthetic organic approaches for the all-racemic 1-aza- α -tocopherol analogs. **Figure 7A** shows the synthetic scheme for compounds **31-38** and **Figure 7B** shows the 20 synthetic scheme for compounds **39-43**.

Figure 8 shows the mean body weights of non-tumor Balb/c female mice at day 11 and day 23 of a maximum tolerated dose study.

Figure 9 shows the comparison of percent mean tumor weight following treatments in MDA MB-435 Human Breast Cancer Cells (**Figure 9A**), DU-145 Human Prostrate Cancer Cells (**Figure 9B**) and HT-29 Human Colon Cancer Cells (**Figure 9C**).

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are given for the purpose of facilitating understanding of the inventions disclosed herein. Any terms not specifically defined should be interpreted according to the common meaning of the term in the art.

As used herein, the term "individual" shall refer to animals and humans.

As used herein, the term "biologically inhibiting" or "inhibition" of the growth of proliferating cells shall include partial or total growth inhibition and also is meant to include decreases in the rate of proliferation or growth of the cells. The biologically inhibitory

dose of the composition of the present invention may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell culture or any other method known to those of
5 ordinary skill in the art.

As used herein, the term "induction of programmed cell death or apoptosis" shall include partial or total cell death with cells exhibiting established morphological and biochemical apoptotic characteristics. The dose of the composition of the present invention
10 that induces apoptosis may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell culture or any other method known to those of ordinary skill in the art.

As used herein, the term "induction of cell cycle arrest"
15 shall include growth arrest due to treated cells being blocked in G0/G1 or G2/M cell cycle phase. The dose of the composition of the present invention that induces cell cycle arrest may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and
20 cell culture or any other method known to those of ordinary skill in the art.

As used herein, the term "induction of cellular differentiation" shall include growth arrest due to treated cells being induced to undergo cellular differentiation, a stage in which cellular proliferation does not occur. The dose of the composition of the present invention that induces cellular differentiation may be determined by assessing the effects of the test element on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell culture or any other method known to those of ordinary skill in the art.

The present invention provides tocopherols, tocotrienols, and other chroman derivatives with or without derivatives of saturated phytyl or unsaturated isoprenyl side chains and analogs; e.g., azo- and thiol- analogs, thereof. Utilizing ethers and several other chemical linkages to attach different moieties to tocopherol, tocotrienol and other chroman derivatives, novel anti-cancer compounds, for *in vivo* use, are produced. The general structures of the novel compounds of the present invention are shown in Figure 1, the preferred compounds are listed in Figure 2 and possible routes for their syntheses are provided in Figures 3-7. The novel features of these molecules include chemical functionalization of positions $R^1 - R^5$ of the chroman structure, and chemical functionalization of the phytyl and isoprenyl side chains,

particularly compounds based on tocopherols and tocotrienols (Figure 1). Additionally, compounds with heteroatom substitutions (N or S) for the chroman ring oxygen are presented (Figure 7). Particularly preferred compounds include 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (1), 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)propionic acid (2), 2,5,8-trimethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (7), 2,7,8-trimethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (8), 2,8-dimethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (9), 2-(N,N-(carboxymethyl)-2(2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (12), 2,5,7,8-tetramethyl-(2RS-(4RS,8RS,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (15), 2,5,7,8-tetramethyl-2R-(2RS,6RS,10-trimethylundecyl)chroman-6-yloxy)acetic acid (17), 3-(2,5,7,8-tetramethyl-(2R-(4R,8,12-trimethyltridecyl)chroman-6-yloxy)propyl-1-ammonium chloride (19), 2,5,7,8-tetramethyl-(2R-(4r,8R,12-trimethyltridecyl)chroman-3-ene-6-yloxy)acetic acid (20), 2-(2,5,7,8-tetramethyl-(2R-(4R,8,12-trimethyltridecyl)chroman-6-yloxy)triethylammonium sulfate (21), 6-(2,5,7,8-tetramethyl-(2R-(4R,8,12-trimethyltridecyl)chroman)acetic acid (22), 2,5,7,8,-

tetramethyl-(2R-(heptadecyl)chroman-6-yloxy) acetic acid (25),
 2,5,7,8,-tetramethyl-2R-(4,8,-dimethyl-1,3,7 E:Z nonotrien)chroman-6-
 yloxy) acetic acid (26), E,Z,RS,RS-(phytyltrimethylbenzenethiol-6-
 yloxy)acetic acid (27), 1-aza- α -tocopherol-6-yloxyl-acetic acid (39),
 5 1-aza- α -tocopherol-6-yloxyl-methyl acetate (40), 1-aza-N-methyl- α -
 tocopherol-6-yloxyl-methyl acetate (41), 1-aza-N-methyl- α -
 tocopherol-6-yloxyl- acetic acid (42), 6-(2,4-
 Dinitrophenylazo(2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-
 1,2,3,4-tetrahydroquinoline (43).

10 The pharmacodynamically designed compounds of the
 present invention have an improved therapeutic index and are potent
 inhibitors of cancer cell growth; i.e., they demonstrate high antitumor
 activity with minimal side effects. These compounds, which can not be
 readily degraded since there are no known etherases in mammals, may
 15 be used in the treatment of cancers and disorders involving excess cell
 proliferation, as well as for cells that accumulate in numbers due to
 suppressed cell killing mechanisms, with minimal side effects. The
 compounds of the present invention inhibit cancer cell growth by
 induction of apoptosis and DNA synthesis arrest. Induction of
 20 apoptosis by these compounds is mediated by activation of the TGF- β ,
 stress kinase, and Fas/Fas ligand signaling pathways. Induction of

apoptosis by other pathways, for example, ceramide production, are not excluded. These growth inhibitory properties allow these compounds to be used in the treatment of proliferative diseases, including cancers of different cell types and lineages, non-neoplastic
5 hyperproliferative diseases, and disorders with defects in apoptotic signaling pathways. Several of the compounds of the present invention are both strong inducers of apoptosis and strong inhibitors of DNA synthesis arrest of tumor cells representing different cellular lineages.

The therapeutic use of the compounds of the present
10 invention in treatment of cancers and other diseases and disorders involving excess cell proliferation or failure of cells to die is illustrated. The novel derivatives (Tables 1 and 2) were shown at EC_{50} concentrations to induce apoptosis of human breast cancer cells (MDA MB 435, MDA MB 231, and MCF-7 breast cancer cells), human prostate
15 cancer cells (PC-3, DU-145 and LnCaP), human ovarian tumor cells (C-170), human cervical tumor cells (ME-180), human endometrial cells (RL-95-2), human lymphoid cells (myeloma, Raji, Ramos, Jurkat, and HL-60), colon cancer cells (HT-29 and DLD-1) and lung cancer cells (A-549). The novel derivatives were shown to not induce apoptosis of
20 normal human mammary epithelial cells (HMECs) and immortalized but non-tumorigenic MCF-10A mammary cells.

These novel compounds and methods of the present invention may be used to treat neoplastic diseases and non-neoplastic diseases. Representative examples of neoplastic diseases are ovarian cancer, cervical cancer, endometrial cancer, bladder cancer, lung cancer, cervical cancer, breast cancer, prostate cancer, testicular cancer, gliomas, fibrosarcomas, retinoblastomas, melanomas, soft tissue sarcomas, osteosarcomas, colon cancer, carcinoma of the kidney, pancreatic cancer, basal cell carcinoma, and squamous cell carcinoma. Representative examples of non-neoplastic diseases are selected from the group consisting of psoriasis, benign proliferative skin diseases, ichthyosis, papilloma, restinosis, scleroderma and hemangioma.

The compounds and methods of the present invention may be used to treat non-neoplastic diseases that develop due to failure of selected cells to undergo normal programmed cell death or apoptosis. Representative examples of diseases and disorders that occur due to the failure of cells to die are autoimmune diseases. Autoimmune diseases are characterized by immune cell destruction of self cells, tissues and organs. A representative group of autoimmune diseases includes autoimmune thyroiditis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, dermatitis herpetiformis, celiac disease,

and rheumatoid arthritis. This invention is not limited to autoimmunity, but includes all disorders having an immune component, such as the inflammatory process involved in cardiovascular plaque formation, or ultra violet radiation induced skin
5 damage.

The compounds and methods of the present invention may be used to treat disorders and diseases that develop due to virus infections. Representative examples of diseases and disorders that occur due to virus infections are human immunodeficiency viruses
10 (HIV). Since these compounds are working on intracellular signaling networks, they have the capacity to impact on any type of external cellular signal such as cytokines, viruses, bacteria, toxins, heavy metals, etc.

The methods of the present invention may be used to treat
15 any animal. Most preferably, the methods of the present invention are useful in humans.

Generally, to achieve pharmacologically efficacious cell killing and anti-proliferative effects, these compounds and analogs thereof may be administered in any therapeutically effective dose.
20 Preferably, the structurally modified tocopherols and tocotrienols and analogs are administered in a dose of from about 0.1 mg/kg to about

100 mg/kg. More preferably, the structurally modified tocopherols and tocotrienols and analogs are administered in a dose of from about 1 mg/kg to about 10 mg/kg.

Administration of the compositions of the present invention
5 may be by topical, intraocular, parenteral, oral, intranasal, intravenous, intramuscular, subcutaneous, or any other suitable means. The dosage administered is dependent upon the age, clinical stage and extent of the disease or genetic predisposition of the individual, location, weight, kind of concurrent treatment, if any, and
10 nature of the pathological or malignant condition. The effective delivery system useful in the method of the present invention may be employed in such forms as capsules, tablets, liquid solutions, suspensions, or elixirs, for oral administration, or sterile liquid forms such as solutions, suspensions or emulsions. For topical use it may be
15 employed in such forms as ointments, creams or sprays. Any inert carrier is preferably used in combination with suitable solubilizing agents, such as saline, or phosphate-buffered saline, or any such carrier in which the compounds used in the method, such as ethanol, acetone, or DMSO, of the present invention have suitable solubility
20 properties.

There are a wide variety of pathological cancerous and noncancerous cell proliferative conditions and cell accumulations due to absence of normal cellular death for which the compositions and methods of the present invention will provide therapeutic benefits.

5 These pathological conditions may occur in almost all cell types capable of abnormal cell proliferation or defective in programmed cell death mechanisms. Among the cell types which exhibit pathological or abnormal growth or abnormal death are (1) fibroblasts, (2) vascular endothelial cells and (3) epithelial cells. It can be seen from the above
10 that the methods of the present invention is useful in treating local or disseminated pathological conditions in all or almost all organ and tissue systems of individuals.

It is specifically contemplated that pharmaceutical compositions may be prepared using the novel chroman-based
15 compounds and derivatives and analogs thereof of the present invention. In such a case, the pharmaceutical composition comprises the novel compounds of the present invention and a pharmaceutically acceptable carrier. A person having ordinary skill in this art would readily be able to determine, without undue experimentation, the
20 appropriate dosages and routes of administration of the compounds and analogs of the present invention.

Thus the present invention is directed toward the design and effective use of novel agents that can specifically target cancer cells and either down-regulate growth stimulatory signals, up-regulate growth inhibitory signals, down-regulate survival signals and/or up-regulate death signals. More specifically, this invention creates and characterizes novel agents that activate growth inhibitory factors, trigger death signaling pathways, and inhibit DNA synthesis.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

15 Synthetic Organic Methodology

The synthesis of a variety of tocopherol, tocotrienol, and other chroman derivatives with or without derivatives of saturated phytyl or unsaturated isoprenyl side chains or analogs thereof is possible via structural modification of the chroman ring system (Figures 3-6) and heteroatom substitutions (N or S) for the chroman ring oxygen (Figure 7A and 7B). The structural variables R^1 , R^2 , R^3 , R^4 ,

R⁵, and X illustrate the groups on the chroman group that are modified and Y represents either oxygen, or heteroatom substitutions (N or S) for the chroman ring oxygen. Using alkylation chemistry, a large number of compounds containing different R¹ groups can be synthesized, particularly when X is oxygen. After alkylation, further chemical modification of the R¹ groups permits the synthesis of a wide range of novel compounds. Bromination of the benzylic methyl groups of the chroman group provide intermediates that permit variation of the R², R³ and R⁴ groups. Variation of group R⁵ is also possible, particularly when starting from the commercially available 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. When a heteroatom substitution of nitrogen for the chroman ring oxygen occurs, than nitrogen may be substituted with R⁶ which is hydrogen or methyl. Variation of X to groups other than oxygen, which is the identity of X in tocopherols and tocotrienols, can be accomplished using palladium chemistry (for X = CH₂) and nucleophilic aromatic substitution (for X = N or S). Other possible modifications to the chroman structure include unsaturation at the 3-4 positions and ring contraction to produce a five-membered furanyl ring.

Reagents employed were either commercially available or prepared according to a known procedure. Anhydrous CH₂Cl₂ and THF

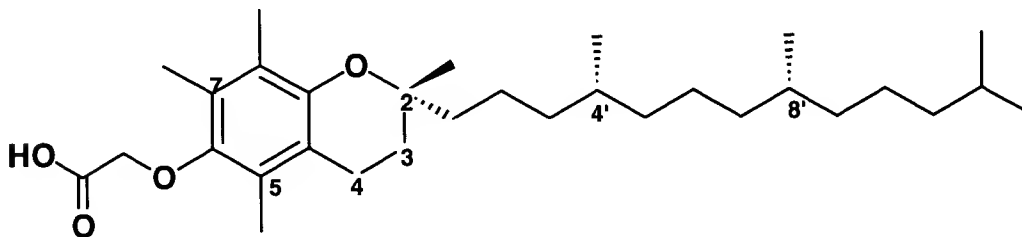
were obtained by distillation. All other solvents used were reagent. Anhydrous reaction conditions were maintained under a slightly positive argon atmosphere in oven-dried glassware. Silica gel chromatography was performed using 230-400 mesh silica purchased from EM Science. Routine ^1H - and ^{13}C -NMR spectra were obtained on a Varian Unity spectrometer at 300.132 MHz and 75.033 MHz frequencies, respectively. NMR spectra were referenced to TMS (0 ppm) or to the isotopic impurity peak of CDCl_3 (7.26 and 77.0 ppm for ^1H and ^{13}C , respectively). High resolution electron impact ionization mass spectroscopy was performed by the Mass Spectrometry Center at The University of Texas at Austin.

EXAMPLE 2

15

Synthesis and Characterization of Novel Tocopherol Compounds

2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)
acetic acid (1)



A solution of R,R,R- α -tocopherol (0.5 g, 1.16 mmol) in N,N-dimethylformamide (20 mL) was treated with methyl bromoacetate (3.4 g, 8.3 mmol) and an excess of powdered NaOH (1.2 g, 30 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and extracted with diethyl ether (3 x 30 mL). The combined ether layers were washed with H₂O (3 x 30 mL) and brine (1 x 30 mL), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with 19% (v/v) EtOAc and 2% acetic acid in hexanes. The resulting yellow liquid was dissolved in diethyl ether (30 mL), washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and then dried with Na₂SO₄. The resulting solution was concentrated to a light yellow oil and dried in vacuo for 48 h. This yielded 1 as a waxy, off-white solid (0.50 g, 88%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.07, 2.14, 2.16 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.59 (t, J = 6.6 Hz, 2H, 4-CH₂), 4.34 (s, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 69.2 (OCH₂),

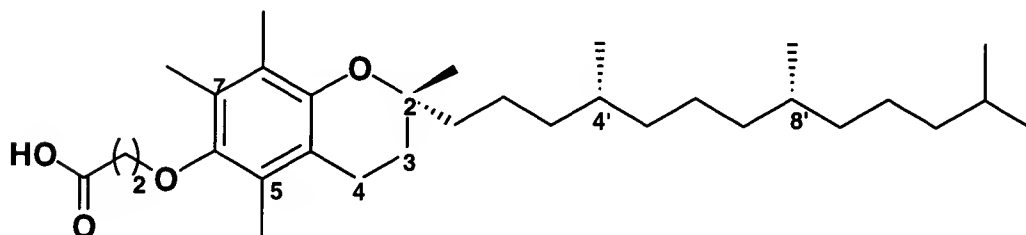
75.0 (2-C), 117.8, 123.2, 125.4, 127.3 (aryl C), 147.0, 148.5 (aryl C-O), 173.7 (COOH); HRMS (CI, m/z): 489.394374 (M + H⁺, Calc. for C₃₁H₅₃O₄ 489.394386). All assignments were confirmed using HMQC, DEPT-135, and ¹H-NOSEY.

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2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy) propionic acid (2)

The compounds 2-6 are synthesized in a manner identical to the synthesis of 1 using the appropriate bromoalkanoic acids.

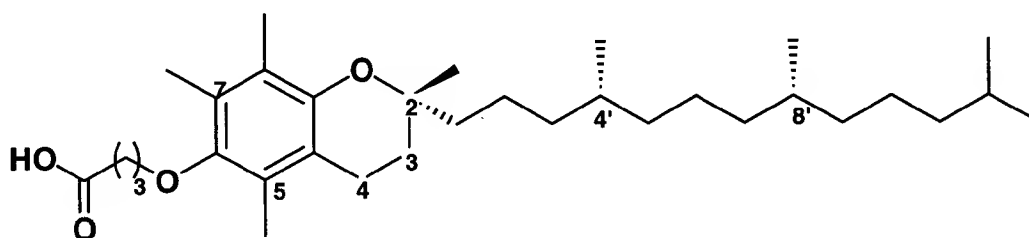
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(89% yield). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.09, 2.14, 2.19 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.59 (t, J = 6.6 Hz, 2H, 4-CH₂), 2.85 (t, J = 6.4 Hz, 2H, CH₂COOH), 3.96 (t, J = 6.4 Hz, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 35.1, 37.3, 37.4, 37.5, 39.4,

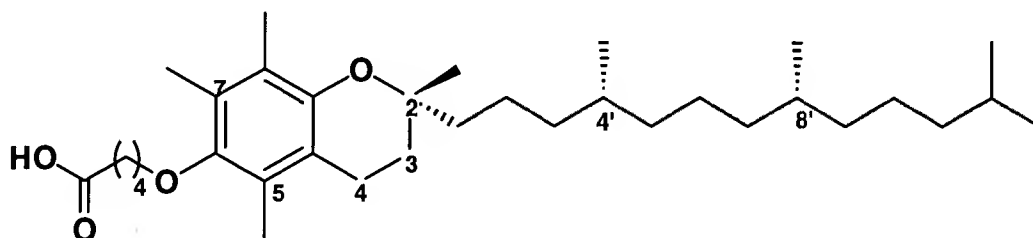
40.0 (CH₂), 67.5 (OCH₂), 74.8 (2-C), 117.5, 122.9, 125.8, 127.8 (aryl C), 147.6, 148.0 (aryl C-O), 177.1 (COOH); HRMS (CI, m/z): 503.408610 (M + H⁺, Calc. for C₃₂H₅₅O₄ 503.410036).

5 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)butyric acid (3)



(85% yield). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 26H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.14, 2.17, 2.21 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.62 (t, J = 6.6 Hz, 2H, 4-CH₂), 2.72 (t, J = 7.2 Hz, 2H, CH₂COOH), 3.74 (t, J = 6.1 Hz, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.9 (2a-CH₃), 24.4, 24.8, 25.3 (CH₂), 28.0 (CH), 30.9, 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 71.3 (OCH₂), 74.8 (2-C), 117.5, 122.9, 125.7, 127.7 (aryl C), 147.8, 147.9 (aryl C-O), 178.9 (COOH); HRMS (CI, m/z): 516.424374 (M + H⁺, Calc. for C₃₃H₅₇O₄ 516.424386).

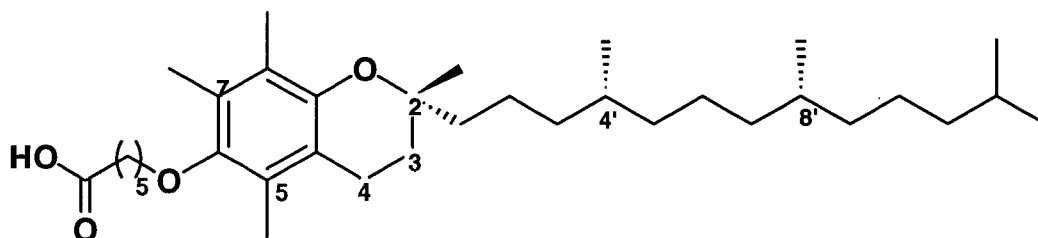
2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)
valeric acid (4)



5

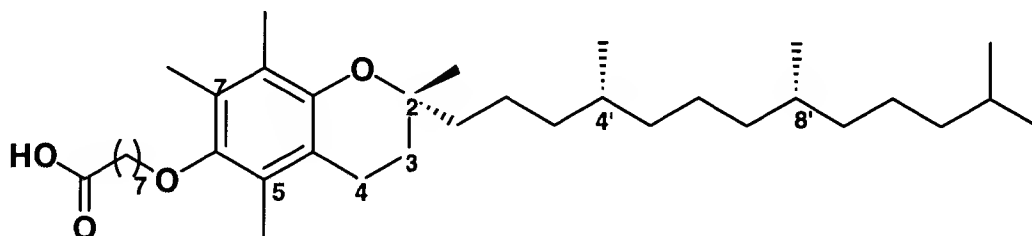
(90% yield). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 28H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.09, 2.14, 2.18 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.49 (t, J = 6.8 Hz, 2H, CH₂COOH), 2.59 (t, J = 6.6 Hz, 2H, 4-CH₂), 3.68 (t, J = 5.5 Hz, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0, 21.4 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 30.0 (CH₂), 31.2 (3-CH₂), 32.7, 32.8 (CH), 35.8, 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 72.2 (OCH₂), 74.9 (2-C), 117.8, 123.2, 125.4, 127.3 (aryl C), 147.6, 148.3 (aryl C-O), 178.7 (COOH); HRMS (CI, m/z): 530.433514 (M + H⁺, Calc. for C₃₄H₅₉O₄ 530.433516).

2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy
hexanoic acid (5)



- 5 (77% yield). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 30H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.08, 2.12, 2.16 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.32 (t, J = 6.5 Hz, 2H, CH₂COOH), 2.57 (t, J = 6.6 Hz, 2H, 4-CH₂), 3.64 (t, J = 5.5 Hz, 2H, OCH₂); ¹³C-NMR
- 10 (CDCl₃, ppm): 11.8, 11.9, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.6, 24.8, 25.7 (CH₂), 28.0 (CH), 30.0 (CH₂), 31.3 (3-CH₂), 32.7, 32.8 (CH), 34.0, 37.3, 37.3, 37.4, 39.3, 40.0 (CH₂), 72.6 (OCH₂), 74.7 (2-C), 117.4, 122.7, 125.4, 127.8 (aryl C), 147.6, 148.2 (aryl C-O), 179.6 (COOH);
- 15 HRMS (CI, m/z): 545.457026 (M + H⁺, Calc. for C₃₅H₆₁O₄ 545.456986).

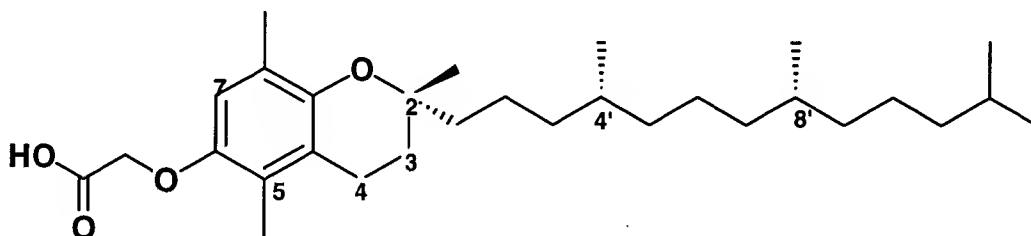
2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy
octanoic acid (6)



- 5 (91% yield). $^1\text{H-NMR}$ (CDCl_3/TMS , ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'- CH_3), 1.0 - 1.6 (m, 34H, 4'-, 8'-, 12'- CH , 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'- CH_2 , 2a- CH_3), 1.81 (m, 2H, 3- CH_2), 2.08, 2.11, 2.16 (3 x s, 9H, 5a-, 7a-, 8a- CH_3), 2.36 (m, 2H, CH_2COOH), 2.58 (t, J = 6.6 Hz, 2H, 4- CH_2), 3.62 (t, J = 5.5 Hz, 2H, OCH_2); $^{13}\text{C-NMR}$ (CDCl_3 ,
10 ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a- CH_3), 19.6, 19.7 (CH_3), 20.6, 21.0 (CH_2), 22.6, 22.7 (CH_3), 23.8 (2a- CH_3), 24.4, 24.6, 24.8, 25.1, 25.7, 26.6 (CH_2), 28.0 (CH), 30.0 (CH_2), 31.3 (3- CH_2), 32.7, 32.8 (CH), 34.0, 37.3, 37.3, 37.4, 39.3, 40.0 (CH_2), 72.7 (OCH_2), 74.6 (2-C), 117.6, 122.8, 125.5, 127.6 (aryl C), 147.5, 148.3 (aryl C-O), 179.4 (COOH);
15 HRMS (CI, m/z): 573.484396 ($\text{M} + \text{H}^+$, Calc. for $\text{C}_{37}\text{H}_{65}\text{O}_4$ 573.488286).

2,5,8-trimethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)

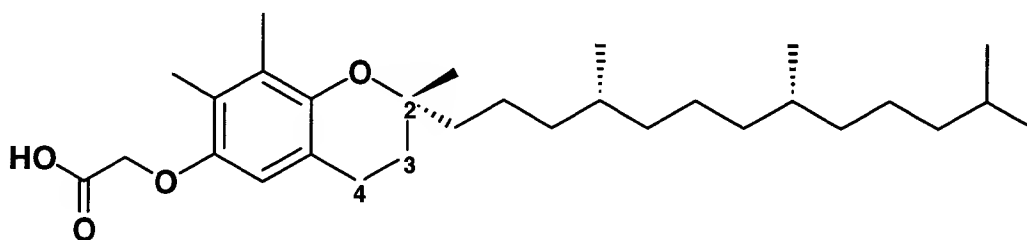
acetic acid (7)



5 A solution of R,R,R- α -tocopherol (75 mg, 0.18 mmol) in
N,N-dimethylformamide (2 mL) was treated with methyl bromoacetate
(0.4 g, 2.8 mmol) and an excess of powdered NaOH (0.5 g, 12.5
mmol). The resulting yellow slurry was stirred vigorously for 24 h at
room temperature. The reaction was acidified with 5 N HCl and
10 extracted with diethyl ether (3 x 10 ml). The combined ether layers
were washed with H₂O (3 x 10 ml) and brine (1 x 10 ml), and then
dried with Na₂SO₄. The ether solution was concentrated to a yellow oil
that was purified by silica gel chromatography eluting with 19% (v/v)
EtOAc and 2% acetic acid in hexanes. The resulting yellow liquid was
15 dissolved in diethyl ether (30 ml), washed with H₂O (3 x 10 mL) and
brine (1 x 10 mL), and then dried with Na₂SO₄. The resulting solution
was concentrated to a light yellow oil and dried in vacuo for 48 h. This
yielded 7 as a waxy, off-white solid (80 mg, 97%). ¹H-NMR

(CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.12, 2.14 (2 x s, 6H, 5a-, 8a-CH₃), 2.61 (t, J = 6.6 Hz, 2H, 4-CH₂), 4.59 (s, 2H, OCH₂), 6.53 (s, 1H, aryl CH); ¹³C-NMR (CDCl₃, ppm): 11.2, 16.1 (5a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.7, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 27.9 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.2, 37.4, 37.5, 39.4, 40.0 (CH₂), 66.8 (OCH₂), 74.8 (2-C), 113.8, 120.7, 123.1, 127.3 (aryl C), 147.1, 148.2 (aryl C-O), 175.3 (COOH); HRMS (CI, m/z): 475.377840 (M + H⁺, Calc. for C₃₀H₅₁O₄ 475.378736).

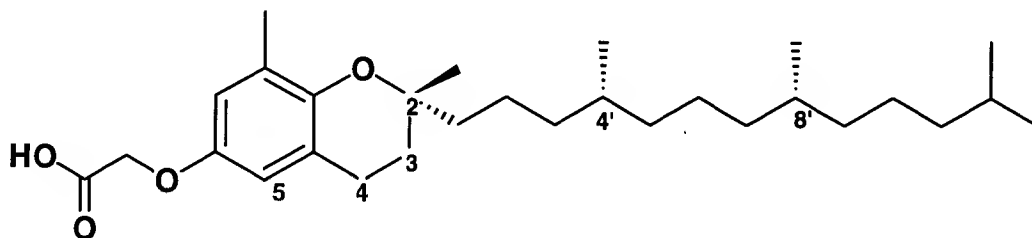
2,7,8-trimethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (8)



15 A solution of R,R,R- α -tocopherol (100 mg, 0.24 mmol) in N,N-dimethylformamide (5 mL) was treated with methyl bromoacetate (1.1 g, 7.4 mmol) and an excess of powdered NaOH (1.0 g, 25 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and extracted

with diethyl ether (3 x 10 ml). The combined ether layers were washed with H₂O (3 x 10 ml) and brine (1 x 10 ml), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with 19% (v/v) EtOAc and 2% acetic acid in hexanes. The resulting yellow liquid was dissolved in diethyl ether (30 ml), washed with H₂O (3 x 10 mL) and brine (1 x 10 mL), and then dried with Na₂SO₄. The resulting solution was concentrated to a light yellow oil and dried in vacuo for 48 h. This yielded 8 as a waxy, off-white solid (110 mg, 97%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.12, 2.19 (2 x s, 6H, 7a-, 8a-CH₃), 2.61 (t, J = 6.6 Hz, 2H, 4-CH₂), 4.59 (s, 2H, OCH₂), 6.39 (s, 1H, aryl CH); ¹³C-NMR (CDCl₃, ppm): 11.9, 12.0 (7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.7, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 27.9 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.2, 37.4, 37.5, 39.4, 40.0 (CH₂), 66.6 (OCH₂), 75.7 (2-C), 110.6, 117.7, 125.0, 126.3 (aryl C), 146.9, 148.7 (aryl C-O), 175.0 (COOH); HRMS (CI, m/z): 475.377962 (M + H⁺, Calc. for C₃₀H₅₁O₄ 475.378736).

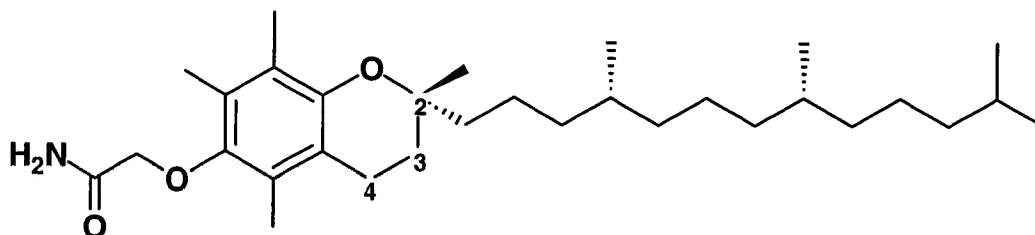
2,8-dimethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy) acetic acid (9)



A solution of R,R,R- α -tocopherol (100 mg, 0.25 mmol) in
5 N,N-dimethylformamide (5 mL) was treated with methyl bromoacetate
(1.1 g, 7.4 mmol) and an excess of powdered NaOH (1.0 g, 25 mmol).
The resulting yellow slurry was stirred vigorously for 24 h at room
temperature. The reaction was acidified with 5 N HCl and extracted
with diethyl ether (3 x 10 ml). The combined ether layers were washed
10 with H₂O (3 x 10 ml) and brine (1 x 10 ml), and then dried with Na₂SO₄.
The ether solution was concentrated to a yellow oil that was purified by
silica gel chromatography eluting with 19% (v/v) EtOAc and 2% acetic
acid in hexanes. The resulting yellow liquid was dissolved in diethyl
ether (30 ml), washed with H₂O (3 x 10 mL) and brine (1 x 10 mL), and
15 then dried with Na₂SO₄. The resulting solution was concentrated to a
light yellow oil and dried in vacuo for 48 h. This yielded 9 as a waxy,
off-white solid (111 mg, 98%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m,
12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,
2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.15 (s,

3H, 8a-CH₃), 2.71 (t, J = 6.6 Hz, 2H, 4-CH₂), 4.59 (s, 2H, OCH₂), 6.48 (d, J = 3.0 Hz, 1H, aryl CH), 6.61 (d, J = 3.0 Hz, 1H, aryl CH); ¹³C-NMR (CDCl₃, ppm): 16.2 (8a-CH₃), 19.6, 19.7 (CH₃), 21.0 (CH₂), 22.6, 22.7 (CH₃), 24.0 (2a-CH₃), 24.4, 24.8 (CH₂), 27.9 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.2, 37.4, 37.5, 39.4, 40.0 (CH₂), 65.7 (OCH₂), 75.8 (2-C), 112.3, 115.6, 121.1, 127.5 (aryl C), 147.2, 149.9 (aryl C-O), 174.8 (COOH); HRMS (CI, m/z): 460.3552022 (M + H⁺, Calc. for C₃₀H₅₁O₄ 460.355262).

10 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)acetamide (10)



A solution of 1 (0.1 g, 0.2 mmol) in CH₂Cl₂ (5 mL) was treated with N-hydroxysuccinimide (26 mg, 0.23 mmol) and dicyclohexylcarbodiimide (46 mg, 0.23 mmol). After 2 min, a white precipitate formed. The resulting suspension was stirred for 2 h. The reaction stirred for an additional 6 h. The reaction mixture was cooled to -30 °C and filtered. The filtrate was concentrated and the resulting colorless oil was purified by silica gel chromatography eluting with

EtOAc (35%, v/v) in hexanes. This yielded a white solid (75 mg, 76%).

¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃),

1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂,

2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.10, 2.12, 2.16 (3 x s, 9H, 5a-, 7a-, 8a-

5 CH₃), 2.59 (t, J = 6.6 Hz, 2H, 4-CH₂), 4.19 (s, 2H, OCH₂), 6.36, 6.92 (2

x broad, 2H, NH); ¹³C-NMR (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-,

8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-

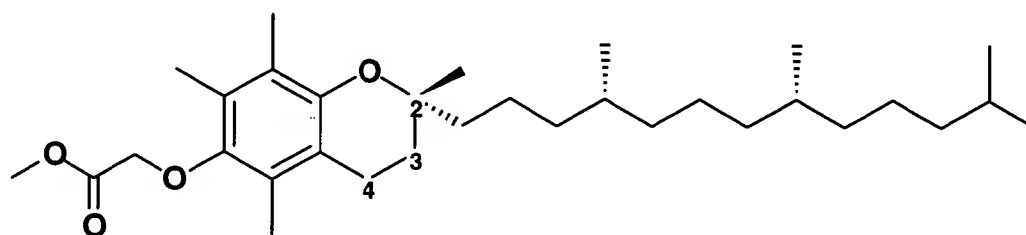
CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3,

37.4, 37.5, 39.4, 40.0 (CH₂), 70.9 (OCH₂), 74.9 (2-C), 117.8, 123.3.

10 125.4, 127.3 (aryl C), 146.5, 148.4 (aryl C-O), 172.1 (COOH); HRMS

(CI, m/z): 488.409341 (M + H⁺, Calc. for C₃₁H₅₄NO₃ 488.410370).

Methyl 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-
ylloxy) acetate (11)

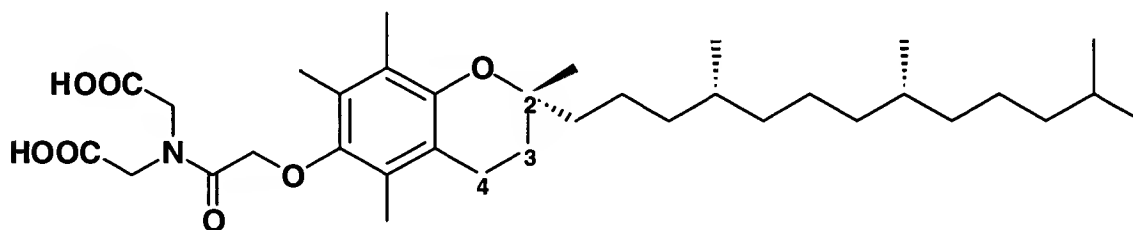


15 A solution of 1 (0.1 g, 0.2 mmol) in CH₂Cl₂ (5 mL) was treated with N,N-dimethylaminopyridine (26 mg, 0.23 mmol), methanol (1 mL) and dicyclohexylcarbodiimide (46 mg, 0.23 mmol). After 2 min, a white precipitate formed. The resulting suspension was

stirred for 6 h. The reaction mixture was cooled to $-30\text{ }^{\circ}\text{C}$ and filtered. The filtrate was concentrated and the resulting colorless oil was purified by silica gel chromatography eluting with EtOAc (40%, v/v) in hexanes. This yielded a white solid (82 mg, 80%). $^1\text{H-NMR}$ (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.10, 2.16, 2.20 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.59 (t, $J = 6.6\text{ Hz}$, 2H, 4-CH₂), 3.85 (s, 3H, OCH₃), 4.32 (s, 2H, OCH₂); $^{13}\text{C-NMR}$ (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 50.2 (OCH₃), 69.8 (OCH₂), 74.9 (2-C), 117.6, 123.0, 125.6, 127.5 (aryl C), 147.6, 148.2 (aryl C-O), 169.8 (COOH); HRMS (CI, m/z): 503.408411 ($M + \text{H}^+$, Calc. for C₃₂H₅₅O₄ 503.410036).

15

2-(N,N-(carboxymethyl)-2(2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyl tridecyl)chroman-6-yloxy)acetic acid (12)



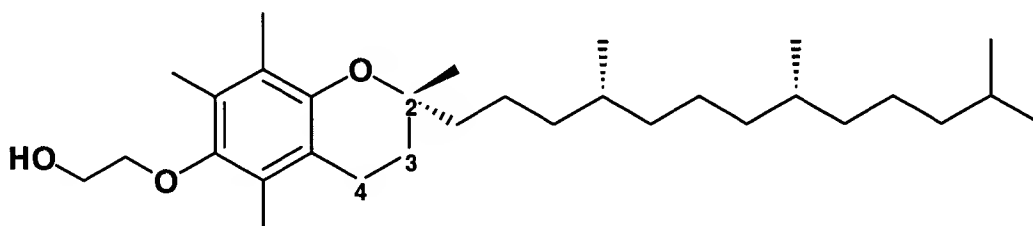
A solution of 1 (0.2 g, 0.4 mmol) in CH_2Cl_2 (5 mL) was treated with diethyl iminodiacetate (77 mg, 0.4 mmol) and O-7-aza benzotriazol-1-yl-N,N,N',N'-tetramethyuroniumhexafluoro phosphate (HATU) (46 mg, 0.23 mmol). After 12 h, the reaction mixture was concentrated to a paste and then purified by silica gel chromatography eluting with EtOAc (30%, v/v) in hexanes. This yielded the desired diester intermediate as colorless oil (150 mg, 55%). $^1\text{H-NMR}$ (CDCl_3/TMS , ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'- CH_3), 1.0 - 1.6 (m, 30H, 4'-, 8'-, 12'- CH , 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'- CH_2 , 2a- CH_3), 1.78 (m, 2H, 3- CH_2), 2.08, 2.13, 2.17 (3 x s, 9H, 5a-, 7a-, 8a- CH_3), 2.58 (t, $J = 6.8$ Hz, 2H, 4- CH_2), 4.19, 4.22 (q, $J = 7.4$ Hz, 4H, OCH_2), 4.30, 4.33, 4.42 (3 x s, 6H, 2 x NCH_2 , OCH_2); $^{13}\text{C-NMR}$ (CDCl_3 , ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a- CH_3), 14.0 (CH_3), 19.6, 19.7 (CH_3), 20.6, 21.0 (CH_2), 22.6, 22.7 (CH_3), 23.8 (2a- CH_3), 24.4, 24.8 (CH_2), 28.0 (CH), 31.2 (3- CH_2), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH_2), 48.1, 49.4 (NCH_2), 61.2, 61.5 (OCH_2), 71.8 (OCH_2), 74.8 (2-C), 117.5, 122.9, 125.6, 127.4 (aryl C), 148.0, 148.1 (aryl C-O), 168.8, 169.0 (CO); MS (CI, m/z): 660 ($\text{M} + \text{H}^+$, Calc. for $\text{C}_{39}\text{H}_{65}\text{NO}_7$ 659.47610).

A solution of the diester intermediate (0.15 g, 0.23 mmol) in ethanol (4 mL) was treated with 1 N NaOH (1 mL). The resulting cloudy mixture was stirred at 70 °C for 15 h. The reaction mixture was

acidified with 1 N HCl and the ethanol was removed in vacuo. The resulting aqueous solution was extracted with CHCl₃ (5 x 20 ml) and the combined organic layers dried with Na₂SO₄. This yielded 12 (0.13 g, 52%) as a white solid. ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.70 (m, 2H, 3-CH₂), 2.01, 2.05, 2.08 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.47 (m, 2H, 4-CH₂), 4.18 (m, 4H, 2 x NCH₂), 4.31 (m, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.5, 11.6, 12.4 (5a-, 7a-, 8a-CH₃), 19.4, 19.5 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 31.2 (3-CH₂), 32.4, 32.5 (CH), 37.0, 37.2, 37.5, 39.1, 40.0 (CH₂), 48.1, 49.4 (NCH₂), 71.1 (OCH₂), 74.8 (2-C), 117.5, 122.9, 125.4, 127.2 (aryl C), 147.8, 148.1 (aryl C-O), 168.8, 169.0 (CO); HRMS (CI, m/z): 604.420882 (M + H⁺, Calc. for C₃₅H₅₈NO₇ 604.421329).

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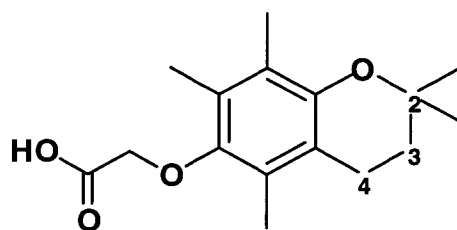
2-(2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy)) ethan-1-ol (13)



A solution of R,R,R- α -tocopherol (0.5 g, 1.16 mmol) in N,N-dimethylformamide (20 mL) was treated with iodoethanol (1.7 g, 10 mmol) and an excess of powdered NaOH (2.5 g, 63 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and extracted with diethyl ether (3 x 30 ml). The combined ether layers were washed with H₂O (3 x 30 ml) and brine (1 x 30 ml), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with 30% (v/v) EtOAc and 2% acetic acid in hexanes. The resulting yellow liquid was dissolved in diethyl ether (30 ml), washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and then dried with Na₂SO₄. The resulting solution was concentrated to a light yellow oil and dried in vacuo for 48 h. This yielded **13** as yellow oil (0.40 g, 73%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.07, 2.14, 2.16 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.59 (t, J = 6.6 Hz, 2H, 4-CH₂), 3.79 (m, 2H, OCH₂), 3.94 (m, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 63.1, 69.2

(OCH₂), 75.0 (2-C), 117.8, 123.4, 126.4, 128.3 (aryl C), 149.2, 149.5 (aryl C-O); MS (CI, m/z): 475 (M + H⁺, Calc. for C₃₁H₅₄O₃ 474.40729).

2-(2,5,7,8-pentamethylchroman-6-yloxy)acetic acid (14)

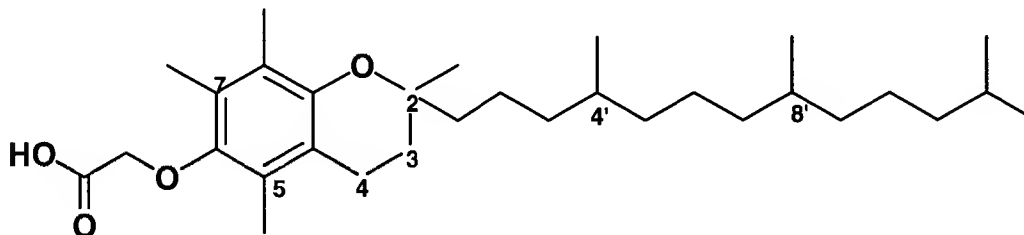


5

A solution of 2,2,5,7,8-pentamethyl-6-chromanol (0.3 g, 1.36 mmol) in N,N-dimethylformamide (20 mL) was treated with methyl bromoacetate (0.8 g, 5.3 mmol) and an excess of powdered NaOH (0.7 g, 18 mmol). The resulting yellow slurry was stirred
10 vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and extracted with diethyl ether (3 x 30 ml). The combined ether layers were washed with H₂O (3 x 30 ml) and brine (1 x 30 ml), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel
15 chromatography eluting with 30% (v/v) EtOAc and 2% acetic acid in hexanes. The resulting yellow liquid was dissolved in diethyl ether (30 ml), washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and then dried with Na₂SO₄. The resulting solution was concentrated to a light yellow oil and dried in vacuo for 48 h. This yielded 14 as a white solid

(0.31 g, 82%). ¹H-NMR (CDCl₃/TMS, ppm): 1.31 (s, 6H, CH₃), 1.81 (t, J = 7.8 Hz, 3-CH₂), 2.10, 2.16, 2.19 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.61 (t, J = 7.8 Hz, 2H, 4-CH₂), 4.39 (s, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.7, 11.8, 12.7 (5a-, 7a-, 8a-CH₃), 20.9, 26.8, 32.7 (alkyl), 69.1, 5 (OCH₂), 72.9 (2-C), 117.5, 123.2, 125.5, 127.3 (aryl), 147.0, 148.6 (O-aryl), 173.8 (COOH); HRMS (CI, m/z): 279.159238 (M + H⁺, Calc. for C₁₆H₂₃O₄ 279.159634).

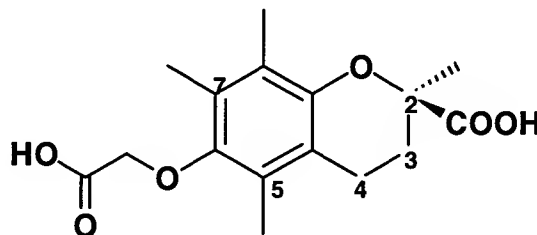
2,5,7,8-tetramethyl-(2RS-(4RS,8RS,12-trimethyltridecyl)chroman-6-yloxy) acetic acid (15)



A solution of all racemic -α-tocopherol (0.5 g, 1.16 mmol) in N,N-dimethylformamide (20 mL) was treated with methyl bromoacetate (3.4 g, 8.3 mmol) and an excess of powdered NaOH (1.2 g, 30 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and

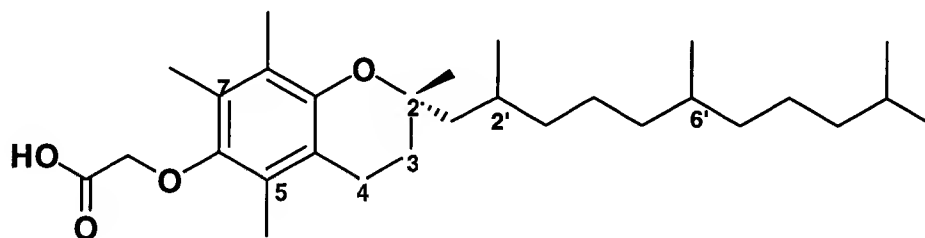
extracted with diethyl ether (3 x 30 ml). The combined ether layers
 were washed with H₂O (3 x 30 ml) and brine (1 x 30 ml), and then dried
 with Na₂SO₄. The ether solution was concentrated to a yellow oil that
 was purified by silica gel chromatography eluting with 19% (v/v) EtOAc
 5 and 2% acetic acid in hexanes. The resulting yellow liquid was
 dissolved in diethyl ether (30 ml), washed with H₂O (3 x 20 mL) and
 brine (1 x 20 mL), and then dried with Na₂SO₄. The resulting solution
 was concentrated to a light yellow oil and dried in vacuo for 48 h. This
 yielded 15 as a waxy, off-white solid (80%). ¹H-NMR (CDCl₃/TMS,
 10 ppm): 0.88 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-,
 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.84 (m, 2H,
 3-CH₂), 2.07, 2.14, 2.16 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.61 (t, J = 6.6
 Hz, 2H, 4-CH₂), 4.34 (s, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 11.5, 11.7,
 12.6 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.3 (CH₂), 22.6, 22.8
 15 (CH₃), 23.8 (2a-CH₃), 24.5, 24.9 (CH₂), 29.0 (CH), 31.6 (3-CH₂), 32.6,
 32.8 (CH), 37.5, 37.8, 37.9, 39.5, 41.0 (CH₂), 69.3 (OCH₂), 75.1 (2-C),
 117.9, 123.3, 125.5, 127.3 (aryl C), 147.0, 148.0 (aryl C-O), 173.9
 (COOH); HRMS (CI, m/z): 489.394375 (M + H⁺, Calc. for C₃₁H₅₃O₄
 489.394383).

2,5,7,8-tetramethyl-(2R-(carboxy)chroman-6-yloxy)) acetic acid (16)



A solution of (-)-(R)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (0.34g, 1.36 mmol) in N,N-dimethylformamide (20 mL) was treated with methyl bromoacetate (0.8 g, 5.3 mmol) and an excess of powdered NaOH (0.7 g, 18 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and extracted with diethyl ether (3 x 30 ml). The combined ether layers were washed with H₂O (3 x 30 ml) and brine (1 x 30 ml), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with 30% (v/v) EtOAc and 2% acetic acid in hexanes. The resulting yellow liquid was dissolved in diethyl ether (30 ml), washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and then dried with Na₂SO₄. The resulting solution was concentrated to light yellow oil and dried in vacuo for 48h. This yielded 16 as a white solid (0.33g, 80%). ¹H-NMR (CDCl₃/TMS, ppm): 1.52 (s, 3H, 2a-CH₃), 2.10 (m, 2H, 3-CH₂), 2.12, 2.16, 2.19 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.56 (t, J = 6.5 Hz, 2H, 4-CH₂), 4.36 (s, 2H, OCH₂).

2,5,7,8-tetramethyl-2R-(2RS,6RS,10-trimethylundecyl)chroman-6-yloxy) acetic acid (17)



A solution of 10g (40mmol) of (-)-(S)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid and 0.5g of p-toluenesulfonic acid monohydrate in 200 ml of methanol was stirred and refluxed for 4hr. After cooling, the solution was diluted with water and extracted with diethyl ether. The combined ether layers were washed with saturated aqueous sodium bicarbonate solution, H₂O, and brine (1 x 30 ml), and then dried with Na₂SO₄. The resulting solution was concentrated and dried in vacuo for 48 h. This yielded 10 g (95%) of methyl (-)-(S)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate as a colorless solid which was used without further purification. ¹H-NMR (CDCl₃/TMS, ppm): 1.52 (s, 3H, 2a-CH₃), 2.10 (m, 2H, 3-CH₂), 2.12, 2.16, 2.19 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.56 (t, J = 6.5 Hz, 2H, 4-CH₂), 3.55 (s, 3H, OCH₃); MS (CI, m/z): 264.422 M + H⁺, Calc. for C₁₅H₂₀O₄ 265.3224.

To a solution of 2g (7.58mmol) of this ester in 7.5ml of N, N-dimethylformamide (DMF) was added 2.6 g (18.8mmol) of

anhydrous granular potassium carbonate followed by 2.3 ml (20 mmol) of benzylchloride. The resulting slurry was stirred at RT for 41 h then poured into 50 ml of water and worked up with ether in the usual way. The product was freed of excess benzyl chloride at 50°
5 under high vacuum. There was obtained 2.69g (100%) of pure (TLC) (-)-(S)-6-benzyloxy-2,5,7,8-tetramethyl-chroman-2 carboxylic acid methyl ester as a yellow solid, m.p. 102-106°. The analytical specimen of this compound was prepared as a colorless solid m.p. 108-109° (from ether/methanol). ¹H-NMR (CDCl₃/TMS, ppm): 1.54 (s, 3H, 2a-
10 CH₃), 2.01 (m, 2H, 3-CH₂), 2.14, 2.17, 2.19 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.51 (t, J = 6.7 Hz, 2H, 4-CH₂), 3.64 (s, 3H, OCH₃), 5.12(s, 2H, 6-OCH₂), 7.15 (m, 5H, ArH); MS (CI, m/z): 355.232 M + H⁺, Calc. for C₂₂H₂₅O₄ 354.448.

A solution of 3.54g (10mmol) of the above ether ester, in
15 20 ml of toluene and 10ml of CH₂Cl₂ was stirred with cooling from dry ice/acetone bath while 12 ml (18 mmol) of 25% disobutylaluminum hydride in toluene (Texas Alkyls) was added dropwise, over 10 min. After stirring at ca. -70° for 30 min, the reaction mixture was cautiously decomposed (-70°) with 10 ml of MeOH. Following the
20 addition of 50 ml of water and 50 ml of 1N aqueous H₂SO₄ solution, the mixture was warmed to RT, and worked up with ether in the usual way

giving 3.2 g (100%) of crude aldehyde [(+) S-6-Benzyloxy-2,5,7,8-tetramethylchroman-2-carbaldehyde] as a viscous oil which was purified by silica gel chromatography eluting with 19% (v/v) EtOAc in hexane. ¹H-NMR (CDCl₃/TMS, ppm) : 1.53 (s, 3H, 2a-CH₃), 2.11 (m, 2H, 3-CH₂),
5 2.24, 2.27, 2.29 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.481 (t, J = 6.7 Hz, 2H, 4-CH₂), 5.19(s, 2H, 6-OCH₂), 7.20 (m, 5H, ArH), 9.6(s, 1H, CHO); MS (CI, m/z): 325.332 M + H⁺, Calc. for C₂₁H₂₄O₃ 324.422.

A solution of 9.6g of pseudoionone was dissolved in 100 ml of 95% ethanol; after 0.68 g of sodium borohydride in ethanol had
10 been added at room temperature, the mixture was stirred for 2 hr and then left standing overnight. The mixture was added to a solution of 2 g of sodium hydroxide in 500 ml of water. The mixture was extracted with ether, and the ether extract was washed with water, dried, and concentrated. The distillation of the residual oil in vacuo gave a
15 colorless oil (pseudoionol); bp 112-120°C/5mmHG. 7.7g (80%).

To a solution of 2.97g of pseudoionol in 10 ml of acetonitrile, there were added, under stirring and while the temperature was kept below 30°C, 4.53g of triphenylphosphine hydrochloride which had been obtained by passing dry hydrogen
20 chloride into a solution of triphenylphosphine in dry ether. After the mixture had been left standing overnight at room temperature, the

acetonitrile was removed under reduced pressure below 50°C. To the residue there were added 4.47 gm of (+) S-6-Benzyloxy-2,5,7,8-tetramethylchroman-2-carbaldehyde in 15 ml of dimethylformamide, and the mixture was stirred. When a clear solution was obtained, 5 sodium methoxide prepared from 0.352 g of sodium and 7 ml of anhydrous methanol was stirred in, drop by drop below 15°C. The reaction mixture was turned red by the ylid formed. After the addition was complete, stirring was continued for 30 min at 10°C; then the mixture was gradually heated to 80°C, when the red color disappeared. 10 The product was poured into 200 ml of 50% aqueous methanol, dried, and concentrated in vacuo. The residual oil was dissolved in 20 ml of ether, and an ethereal solution of mercuric chloride was added until no more precipitate formed. When the precipitate was filtered and the filtrate was washed with water, dried and concentrated, to give 4.7 g of 15 yellow oil were obtained. The crude mixture of cis and trans alkene (MS (CI, m/z): 485.22 , $M + H^+$, Calc. for $C_{34}H_{44}O_2$ 484.7255) was dissolved in 30 ml of ethyl acetate and 0.80 g of 5% palladium on carbon was added, and the mixture was shaken under 40 psi of H_2 for 30 hrs and then filtered through Celilte and rinsed well with ethyl 20 acetate. The filtrate was concentrated and purified by silica gel chromatography eluting with EtOAc in hexane (1:9) to give 2,5,7,8-

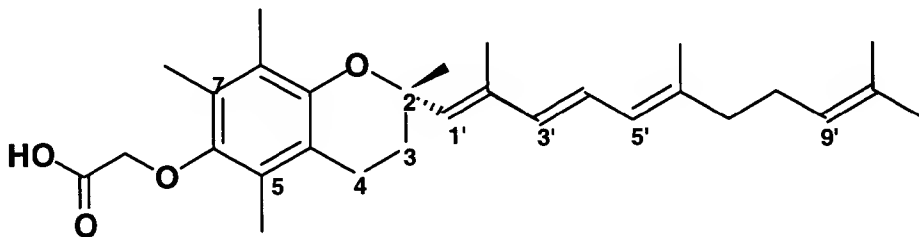
tetramethyl -(2R-(2RS,6RS,10-trimethylundecyl))-6-chromanol (60%
yield) ¹H-NMR (CDCl₃/TMS, ppm): 0.97 (m, 12H, 2a'-, 6a'-, 10a'-, 11'-
CH₃), 1.1 - 1.7 (m, 20H, 2'-, 6'-,10'-CH, 1'-,3'- 4'-,5'-,7'-,8'-,9'-CH₂, 2a-
CH₃), 1.88 (m, 2H, 3-CH₂), 2.17, 2.19, 2.20 (3 x s, 9H, 5a-, 7a-, 8a-
5 CH₃), 2.63 (t, J = 6.7 Hz, 2H, 4-CH₂); (MS (CI, m/z): 403.27 , M + H⁺,
Calc. for C₂₇H₄₆O₂ 402.6632.

A solution of 2,5,7,8-tetramethyl -(2R-(2RS,6RS,10-
trimethylundecyl))-6-chromanol (0.466 g, 1.16 mmol) in N,N-
dimethylformamide (20 mL) was treated with methyl bromoacetate
10 (3.4 g, 8.3 mmol) and an excess of powdered NaOH (1.2 g, 30 mmol).
The resulting yellow slurry was stirred vigorously for 24 h at room
temperature. The reaction was acidified with 5 N HCl and extracted
with diethyl ether (3 x 30 ml). The combined ether layers were washed
with H₂O (3 x 30 ml) and brine (1 x 30 ml) and then dried with Na₂SO₄.
15 The ether solution was concentrated to a yellow oil that was purified by
silica gel chromatography eluting with 19% (v/v) EtOAc and 2% acetic
acid in hexanes. This yielded compound 17 in 76% yield. ¹H-NMR
(CDCl₃/TMS, ppm): 0.97 (m, 12H, 2a'-, 6a'-, 10a'-, 11'-CH₃), 1.2 - 1.7
(m, 20H, 2'-, 6'-,10'-CH, 1'-,3'- 4'-,5'-,7'-,8'-,9'-CH₂, 2a-CH₃), 1.92 (m,
20 2H, 3-CH₂), 2.18, 2.20, 2.23 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.68 (t, J =

6.8 Hz, 2H, 4-CH₂), 4.48 (s, 2H, OCH₂); MS (CI, m/z): 461.44, M + H⁺,
Calc. for C₂₉H₄₈O₄ 460.700.

2,5,7,8-tetramethyl-2R-(2,6,10-trimethyl-1,3,5,9 E:Z decatetraen)

5 chroman-6-yloxy) acetic acid (18)



To a solution of methyl (-)-(S)- 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (20 gms 0.075mole) in 50ml of dry DMF, imidazole (13 gm, 0.1911mole), and tert-butyldimethyl-
10 silylchloride (14 gm, 0.0933 mole) were added. The mixture was stirred at 23°C for 24 hr and then treated with ether and poured into 1N HCl. The organic extracts were dried (brine, Na₂SO₄) and concentrated in vacuo. The crude product was purified by flash chromatography (9:1 hexane :ethyl acetate) to yield 6-[dimethyl (1,1-
15 dimethylethyl) silyl] - 2,5,7,8-tetramethyl-chroman-2-carboxylate (TBS protected methyl ester). . ¹H-NMR (CDCl₃/TMS, ppm) : 0.12(s, 6H). 1.102(s, 9H), 1.18 (s, 3H), 1.48 (s, 3H), 1.645 (s, 3H), 2.07(s, 3H),

2.2 (t, J = 6.5hz 2H), 2.48-2.7 (m, 2H) and 3.72(s,3H, OCH₃) (MS (CI, m/z): 379.32, M + H⁺, Calc. for C₂₁H₃₄O₄ 378.586.

A solution of 3.78 g(10mmol) of the above ether ester, in 20 ml of toluene and 10ml of CH₂Cl₂ was stirred with cooling from dry ice/acetone bath while 12 ml (18 mmol) of 25% disobutylaluminum hydride in toluene (Texas Alkyls) was added dropwise, over 10 min. After stirring at ca. -70° for 30 min, the reaction mixture was cautiously decomposed (-70°) with 10 ml of MeOH. Following the addition of 50 ml of water and 50 ml of 1N aqueous H₂SO₄ solution, the mixture was warmed to RT, and worked up with ether in the usual way giving 3.2g (90%) of crude aldehyde [(+)-6-[dimethyl(1,1-dimethylethyl)silyl]-2,5,7,8-tetramethyl-chroman-2-carbaldehyde] as a viscous oil which was purified by silica gel chromatography eluting with 19% (v/v) EtOAc in hexane. Concentration of the solution followed by drying under vacuo for 48 h yielded TBDS aldehyde (78%) as a solid of mp 66-68°C. ¹H-NMR (CDCl₃/TMS, ppm) : 0.12(s, 6H). 1.1(s, 9H), 1.38 (s, 3H), 1.64 (s, 3H), 2.12 (s, 3H), 2.16(s, 3H), 2.3-2.2 (m, 2H), 2.53 (m, 2H) and 9.82(d, J=1.4Hz, 1H); MS (CI, m/z): 349.40 M + H⁺, Calc. for C₂₀H₃₂SiO₃ 348.560.

To a solution of 2.97 g of pseudoionol in 10 ml of acetonitrile, there were added, under stirring and while the

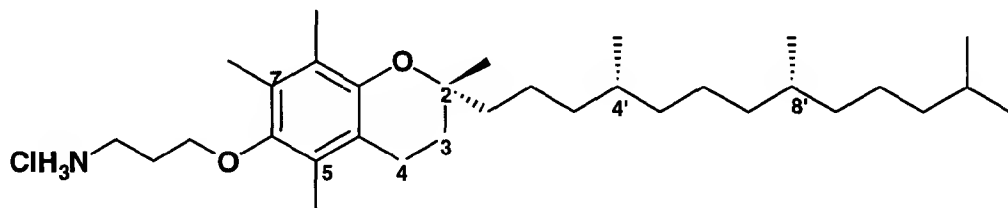
temperature was kept below 30°C, 4.53 g of triphenylphosphine hydrochloride which had been obtained by passing dry hydrogen chloride into a solution of triphenylphosphine in dry ether. After the mixture had then been left standing overnight at room temperature, 5 the acetonitrile was removed under reduced pressure below 50°C. To the residue there were added 4.80 gm of [(+)-6-[dimethyl(1,1-dimethylethyl)silyl]-2,5,7,8-tetramethylchroman-2-carbaldehyde] in 15 ml of dimethylformamide, and the mixture was stirred. When a clear solution was obtained, sodium methoxide prepared from 0.352 g of 10 sodium and 7 ml of anhydrous methanol was stirred in, drop by drop below 15°C. The reaction mixture was turned red by the ylid formed. After the addition was complete, stirring was continued for 30 min at 10°C; then the mixture was gradually heated to 80°C, when the red color disappeared. The product was poured into 200 ml of 50% 15 aqueous methanol, dried, and concentrated in vacuo. The residual oil was dissolved in 20 ml of ether, and an ethereal solution of mercuric chloride was added until no more precipitate formed. When the precipitate was filtered and the filtrate was washed with water, dried and concentrated, to give 4.7 g of yellow oil were obtained. The crude 20 silyl ether mixture of cis and trans alkene was dissolved in THF and tetra-n-butylammoniumfluoride (0.031mole) was added. After being

stirred at 23°C for 40 minutes, the mixture was poured into water and extracted into ether. The ether extract was dried concentrated and purified by silica gel chromatography eluting with EtOAc in hexane (1:9) to give 2,5,7,8-tetramethyl-2R- (2,6,10-trimethyl-1,3,5,9 E:Z
5 decatetraen)- 6-chromanol (68% yield). ¹H-NMR (CDCl₃/TMS, ppm) : 1.28 (s, 3H, 2aCH₃), 1.65(s, 3H), 1.70(s,6H) 1.72 (s,3H), 1.9(m, 6H), 2.18 (s,3H), 2.35 (S, 6H), 2.53 (t, J = 6.6Hz, 2H, 4CH₂), 5.13 - 5.27 (m, 3H) and 6.44(m, 2H) ; MS (CI, m/z): 395.17 M + H⁺, Calc. for C₂₇H₃₈O₂ 394.60.

10 A solution of 2,5,7,8-tetramethyl-2R- (2,6,10-trimethyl-1,3,5,9 E:Z decatetraen)- 6-chromanol (0.457 g, 1.16 mmol) in N,N-dimethylformamide (20 mL) was treated with methyl bromoacetate (3.4 g, 8.3 mmol) and an excess of powdered NaOH (1.2 g, 30 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room
15 temperature. The reaction was acidified with 5 N HCl and extracted with diethyl ether (3 x 30 ml). The combined ether layers were washed with H₂O (3 x 30 ml) and brine (1 x 30 ml), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with 19% (v/v) EtOAc and
20 2% acetic acid in hexanes. The resulting liquid was dissolved in diethyl ether (30 ml), washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and

then dried with Na_2SO_4 . The resulting solution was concentrated and dried in vacuo for 48 h. This yielded compound 18 in 67% yield. ^1H -NMR (CDCl_3/TMS , ppm) : 1.24 (s, 3H, 2a CH_3), 1.63(s, 3H), 1.72(s,6H) 1.74 (s,3H), 1.92(m, 6H), 2.18 (s,3H), 2.29 (S, 6H), 2.43 (t, J = 6.6Hz, 5 2H, 4 CH_2), 4.68 (s,2H, OCH_2), 5.10 - 5.27 (m, 3H) and 6.34(m, 2H) ; MS (CI, m/z): 452.24 M - H^+ , Calc. for $\text{C}_{27}\text{H}_{38}\text{O}_2$ 452.63.

3-(2,5,7,8-tetramethyl-(2R-(4R,8,12-trimethyltridecyl)chroman-6-yloxy)propyl-1-ammonium chloride (19)



10

A solution of 3-bromopropylamine hydrobromide (1.0 g, 4.6 mmol) in a 2:1 dioxane/ H_2O (45 mL) was cooled to 0 °C and treated with K_2CO_3 (6.22 g, 45 mmol) and di-tert-butyl dicarbonate (1.5 g, 6.9 mmol). The reaction was stirred for 15 h while warming to 15 room temperature. The dioxane was removed in vacuo and the remaining aqueous mixture was acidified with 5 N HCl and extracted with ethyl acetate (5 x 25 mL). The combined organic layers were dried with MgSO_4 and yielded 3-bromo-N-(tert-butoxycarbonyl)propylamine as a colorless oil (0.93 g, 93 %). ^1H -NMR

(CDCl₃/TMS, ppm): 1.41 (s 9H, CH₃), 2.02 (quintet, J = 6.4 Hz, 2H, CH₂), 3.23 (m, 2H, NCH₂), 3.41 (t, J = 6.6 Hz, CH₂Br), 4.8 (broad, 1H, NH); ¹³C-NMR (CDCl₃, ppm): 28.3 (CH₃), 30.7, 32.6, 38.9 (CH₂), 79.3 (quaternary C), 155.9 (CO); MS (CI, m/z): 239, 241 (M + H⁺ calc. for C₈H₁₆BrNO₂ 237.03644).

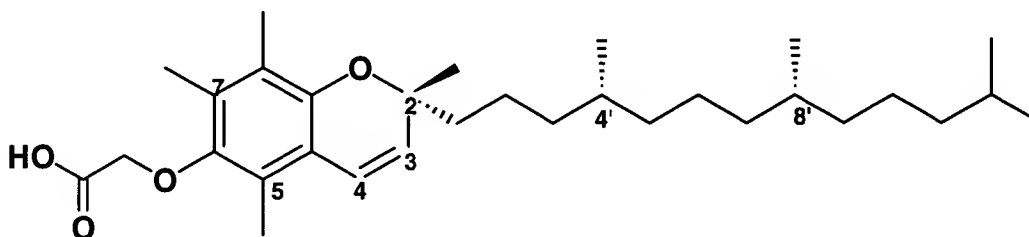
A solution of R,R,R- α -tocopherol (0.5 g, 1.16 mmol) in N,N-dimethylformamide (15 mL) was treated with 3-bromo-N-(tert-butoxycarbonyl)propylamine (0.9 g, 3.8 mmol) and an excess of powdered NaOH (0.32 g, 8 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and extracted with diethyl ether (3 x 30 mL). The combined ether layers were washed with H₂O (3 x 30 mL) and brine (1 x 30 mL), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with EtOAc (10% v/v) in hexanes. This yielded desired ether as a colorless oil (0.45 g, 66%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 33H, 4'-, 8'-, 12'-CH, 1'-, 2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 1.99 (quintet, J = 6.2 Hz, 2H, CH₂), 2.07, 2.14, 2.16 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.59 (t, J = 6.6 Hz, 2H, 4-CH₂), 3.43 (m, 2H, NCH₂), 3.73 (t, J = 5.7 Hz, 2H, OCH₂), 4.34 (s, 2H, OCH₂); ¹³C-NMR (CDCl₃,

ppm): 11.7, 12.0, 12.9 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.7 (2a-CH₃), 24.4, 24.8 (CH₂), 27.9 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.2, 37.4, 37.5, 39.3, 40.1 (CH₂), 70.2 (OCH₂), 74.8 (2-C), 117.5, 122.9, 125.5, 127.5 (aryl C), 147.5, 148.0 (aryl C-O), 156.0 (CO); MS (CI, m/z): 589 M + H⁺, Calc. for C₃₇H₆₅NO₄ 587.49136.

The above N-protected ether (0.1 g, 0.17 mmol) was dissolved 4 N HCl in dioxane (1 mL, 4 mmol) and stirred for 4 h. The dioxane was removed by blowing a stream of argon over the reaction mixture. The resulting material was dried in vacuo for 8 h yielding 19 as a white solid (82 mg, 99%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 33H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 1.99 (quintet, J = 6.2 Hz, 2H, CH₂), 2.07, 2.11, 2.15 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.29 (m, 2H, CH₂), 2.59 (t, J = 6.6 Hz, 2H, 4-CH₂), 3.43 (m, 2H, NCH₂), 3.79 (m, 2H, OCH₂) ¹³C-NMR (CDCl₃, ppm): 11.8, 11.9, 12.7 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.9 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 28.4 (CH₃), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 74.8 (OCH₂), 75.0 (2-C), 117.5, 122.9, 126.0, 127.3 (aryl C), 147.8, 148.0 (aryl C-O);

HRMS (CI, m/z): 487.438887 (M + H⁺, Calc. for C₃₂H₅₇NO₂ 487.438935).

5 2,5,7,8-tetramethyl-(2R-(4R,8R,12-trimethyltridecyl)chroman-3-ene-6-yloxy) acetic acid (20)



A solution of R,R,R,- α -tocopherol acetate (2 g, 4.2mmol) in anhydrous toluene (150 mL) was heated to reflux and then treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.96 g, 4.2 mmol) in 4
10 portions at 1 h intervals. The reaction was refluxed for 24 h. During this time the reaction mixture became a dark red color and then it precipitated a light colored solid. The reaction was cooled to room temperature, filtered, and the filtrate was concentrated. The resulting dark colored oil was purified by silica gel chromatography eluting with
15 ethyl acetate (10%, v/v) in hexanes. This yielded the desired chromene acetate as a colorless oil (1.74g, 88%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 2.07, 2.13, 2.18 (3 x

s, 9H, 5a-, 7a-, 8a-CH₃), 2.35 (s, 3H, CH₃CO-), 5.61, 6.52 (2 x d, J = 10.0 Hz, 2H, CH); ¹³C-NMR (CDCl₃, ppm): 11.5, 11.6, 13.1 (5a-, 7a-, 8a-CH₃), 14.1 (CH₃), 19.6, 19.7 (CH₃), 20.4, 21.4 (CH₂), 22.6, 22.7 (CH₃), 24.4, 24.8 (CH₂), 25.8 (2a-CH₃), 27.9 (CH), 30.8 (3-CH₂), 32.7, 32.8 (CH), 37.2, 37.4, 39.4, 41.0 (CH₂), 60.3 (2-C), 117.6, 119.7, 122.3, 122.6, 128.9, 129.6 (aryl and vinyl C), 141.2, 148.4 (aryl C-O), 169.4 (CO); HRMS (CI, m/z): 471.375799 M + H⁺, Calc. for C₃₁H₅₀O₃ 470.375996.

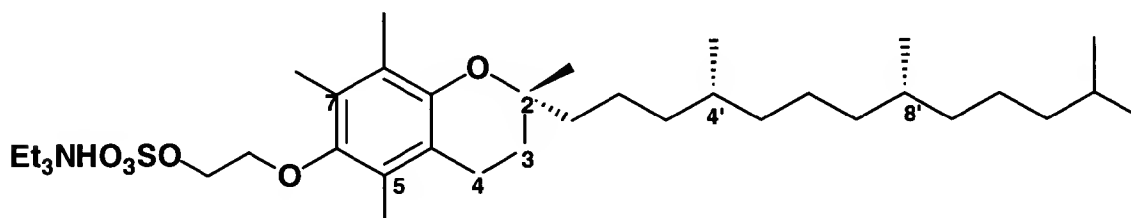
A solution of the chromene acetate (1.0 g, 2.13 mmol) in ethanol (20 mL) was treated with 2 N NaOH (20 mL) and stirred at 60 °C for 90 min. The reaction mixture was cooled, acidified with 5 N HCl, and the ethanol was removed in vacuo. The resulting aqueous solution was extracted with ether and concentrated to a light yellow oil that was purified by silica gel chromatography eluting with ethyl acetate (15%, v/v) in hexanes. This yielded the desired chromene-6-ol intermediate as a colorless oil (0.92 g, 98%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-, 7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 2.14, 2.18, 2.19 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 5.63, 6.55 (2 x d, J = 10.0 Hz, 2H, CH); ¹³C-NMR (CDCl₃, ppm): 10.8, 11.6, 12.4 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 21.3 (CH₂), 22.6, 22.7 (CH₃), 24.4, 24.8 (CH₂), 25.2 (2a-

CH₃), 27.9 (CH), 30.9 (3-CH₂), 32.7, 32.8 (CH), 37.2, 37.4, 37.5, 39.3, 40.5 (CH₂), 50.8 (2-C), 116.2, 117.8, 120.1, 122.3, 123.0, 130.0 (aryl and vinyl C), 144.6, 145.3 (aryl C-O), 169.4 (CO); HRMS (CI, m/z): 428.365275 M + H⁺, Calc. for C₂₉H₄₈O₂ 428.365431.

5 A solution of the chromene-6-ol intermediate (0.9 g, 2.1 mmol) in N,N-dimethylformamide (20 mL) was treated with methyl bromoacetate (3.4 g, 8.3 mmol) and an excess of powdered NaOH (1.2 g, 30 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and
10 extracted with diethyl ether (3 x 30 ml). The combined ether layers were washed with H₂O (3 x 30 ml) and brine (1 x 30 ml), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with 19% (v/v) EtOAc and 2% acetic acid in hexanes. The resulting yellow liquid was
15 dissolved in diethyl ether (30 ml), washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and then dried with Na₂SO₄. The resulting solution was concentrated to a light yellow oil and dried in vacuo for 48 h. This yielded 19 as a colorless (0.90 g, 88%). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-
20 CH, 1'-, 2'-,3'-,5'-,6'-, 7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 2.07, 2.10, 2.19 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 4.37 (s, 2H, OCH₂), 5.62, 6.50 (2 x d, J =

10.0 Hz, 2H, CH); ^{13}C -NMR (CDCl_3 , ppm): 11.3, 11.5, 12.9 (5a-, 7a-, 8a-
 CH₃), 19.6, 19.7 (CH₃), 21.3 (CH₂), 22.6, 22.7 (CH₃), 24.4, 24.8 (CH₂),
 25.6 (2a-CH₃), 27.9 (CH), 30.9 (3-CH₂), 32.7, 32.8 (CH), 37.2, 37.4,
 37.5, 39.3, 40.9 (CH₂), 60.5 (OCH₂), 69.1 (2-C), 118.0, 119.8, 122.8,
 5 122.9, 129.6, 19.8 (aryl and vinyl C), 147.5, 147.8 (aryl C-O), 173.4
 (CO); HRMS (CI, m/z): 487.378731 M + H⁺, Calc. for C₃₁H₅₁O₄
 487.378736.

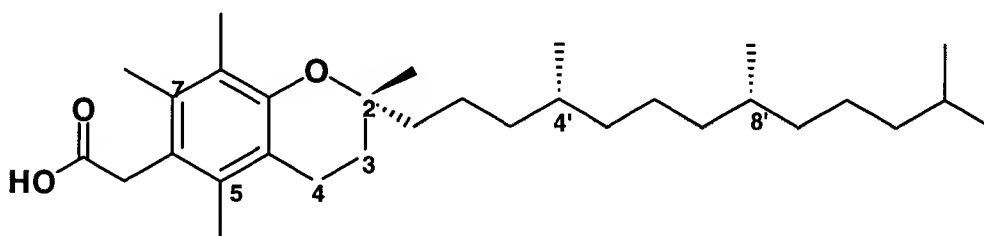
10 2-(2,5,7,8-tetramethyl-(2R-(4R,8,12-trimethyltridecyl)chroman-6-
ylloxy)triethylammonium sulfate (21)



A solution of 2-(2,5,7,8-tetramethyl-(2R-(4R,8R,12-
 trimethyltridecyl)chroman-6-ylloxy))ethan-1-ol (13) (0.1 g, 0.21
 mmol) in anhydrous DMF (2 mL) and pyridine (0.6 mL) was treated
 15 sulfur trioxide-N,N-dimethylformamide complex (0.16 g, 1.0 mmol),
 and the resulting solution was stirred for 24 h. The reaction mixture
 was quenched with 1 N HCl and then extracted with CH₂Cl₂ (5 x 5 mL).
 Gaseous ammonia was bubbled through the CH₂Cl₂ solution for 10 min.
 The resulting solution was concentrated to a yellow paste and purified

by silica gel chromatography eluting with MeOH (10%, v/v) and triethyl amine (2%) in CHCl₃. This yielded 21 as a yellow semi-solid (92 mg, 77%) ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 33H, 4'-, 8'-, 12'-CH, 1'-, 2'-, 3'-, 5'-, 6'-, 7'-, 9'-, 10'-, 11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 1.95 2.01, 2.05 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.45 (t, J = 6.6 Hz, 2H, 4-CH₂), 3.05 (m, 6H, NCH₂), 3.79 (m, 2H, OCH₂), 4.21 (m, 2H, OCH₂); ¹³C-NMR (CDCl₃, ppm): 9.46 (CH₃), 12.4, 12.6, 13.5 (5a-, 7a-, 8a-CH₃), 20.3, 20.4 (CH₃), 21.3, 21.7 (CH₂), 23.3, 23.4 (CH₃), 24.5 (2a-CH₃), 25.1, 25.5 (CH₂), 28.6 (CH), 31.9 (3-CH₂), 33.3, 33.4 (CH), 37.9, 38.1, 40.0, 40.8 (CH₂), 46.9 (NCH₂), 67.4, 71.9 (OCH₂), 75.5 (2-C), 118.3, 123.5, 126.5, 128.3 (aryl C), 148.5 (aryl C-O); HRMS (CI, m/z): 554.364102 M - NH₃, Calc. for C₃₁H₅₄O₆S 554.364119.

15 6-(2,5,7,8-tetramethyl-(2R-(4R,8,12-trimethyltridecyl)chroman) acetic acid (22)



A solution of R,R,R- α -tocopherol (1.0 g, 2.3 mmol) in

anhydrous CH_2Cl_2 (25 mL) was cooled to 0°C . Diisopropylethyl amine (2 mL, 11.6 mmol) was added followed by the dropwise addition of trifluoromethylsulfonic anhydride (5.0 g, 17.7 mmol). The solution turned to a dark immediately and was allowed to warm to room temperature while stirring for 24 h. The reaction was quenched with H_2O and then was extracted with diethyl ether (2 x 100 mL). The combined ether layers were washed with 1 N HCl (50 mL), H_2O (50 mL), brine (50 mL), and then dried with MgSO_4 . The ether solution was concentrated to a yellow oil and purified by silica gel chromatography eluting with ethyl acetate (3%, v/v) in hexane. This yielded the desired triflate intermediate as a yellow oil (1.3 g, quantitative). ^1H -NMR (CDCl_3/TMS , ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'- CH_3), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-, 2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'- CH_2 , 2a- CH_3), 1.81 (m, 2H, 3- CH_2), 2.07, 2.13, 2.21 (3 x s, 9H, 5a-, 7a-, 8a- CH_3), 2.62 (t, $J = 6.6$ Hz, 2H, 4- CH_2); ^{13}C -NMR (CDCl_3 , ppm): 11.9, 13.2, 14.0 (5a-, 7a-, 8a- CH_3), 19.6, 19.7 (CH_3), 20.6, 21.0 (CH_2), 22.6, 22.7 (CH_3), 23.8 (2a- CH_3), 24.4, 24.8 (CH_2), 28.0 (CH), 31.2 (3- CH_2), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH_2), 75.6 (2-C), 118.4, 124.4, 126.7, 128.1 (aryl C), 139.6, 150.9 (aryl C-O); ^{19}F -NMR (CDCl_3 , ppm): -73.52; HRMS (CI, m/z): 563.337803 ($\text{M} + \text{H}^+$, Calc. for $\text{C}_{30}\text{H}_{50}\text{O}_4\text{F}_3\text{S}$ 563.338192).

A solution of the triflate (1.3 g, 2.31 mmol) in anhydrous DMF (23 mL) was treated with LiCl (0.98 g, 4.62 mmol), triphenylphosphine (0.37 g, 1.4 mmol), 2,6-di-tert-butyl-4-methylphenol (2-3 crystals), tributyl(vinyl)tin (0.73 g, 2.31 mmol),
 5 and dichlorobis(triphenylphosphine)-palladium(II) (0.24 g, 0.35 mmol). This mixture was heated to 120 °C and stirred. After 2 h, additional tributyl(vinyl)tin (0.73 g, 2.31 mmol). After 8 h, the reaction was cooled to room temperature and added to a mixture of H₂O (50 mL) and diethyl ether (50 mL). The ether layer was washed
 10 with 1 N HCl (6 x 30 mL) and a saturated solution of KF (6 x 30 mL). The ether solution was dried with Na₂SO₄ and then concentrated to a dark oil. This material was purified by silica gel chromatography eluting with ethyl acetate (3%, v/v) in hexane yielding the 6-vinylchroman intermediate as a clear oil (0.38 g, 38%). ¹H-NMR
 15 (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 1.86 (m, 2H, 3-CH₂), 2.20, 2.24, 2.28 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.62 (t, J = 6.8 Hz, 2H, 4-CH₂), 5.18, 5.56 (2 x dd, J_{gem} = 2.3 Hz, J_{cis} = 11.2 Hz, J_{trans} = 18.7 Hz, 2H, =CH₂), 6.77 (dd, J = 18.7,
 20 11.2 Hz, 1H, CH); ¹³C-NMR (CDCl₃, ppm): 11.9, 16.3, 17.2 (5a-, 7a-, 8a-CH₃), 19.7, 19.8 (CH₃), 20.8, 21.1 (CH₂), 22.6, 22.7 (CH₃), 23.9

(2a-CH₃), 24.5, 24.8 (CH₂), 28.0 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.5, 37.5, 39.4, 40.1 (CH₂), 74.9 (2-C), 116.7, 119.0, 122.0, 129.8, 131.2, 132.8, 136.8 (aryl/vinyl C), 150.9 (aryl C-O); HRMS (CI, m/z): 440.401602 (M + H⁺, Calc. for C₃₁H₅₂O 440.401812).

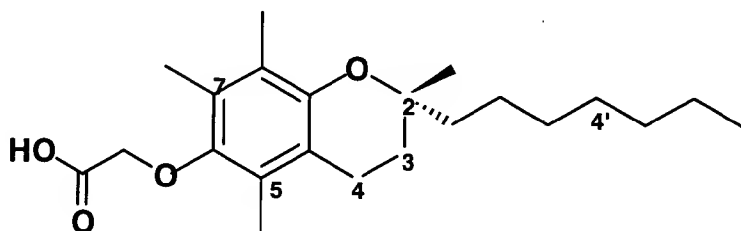
5 A solution of the 6-vinylchroman intermediate (0.12 g, 0.27 mmol) in anhydrous THF (1 mL) was cooled to 0 °C and treated with 9-BBN (0.60 mL, 0.5 M in THF, 0.3 mmol). The reaction mixture was heated to reflux for 8 h. The reaction was quenched with water (1.5 mL) and treated with NaBO₃•4H₂O and the resulting slurry was stirred
10 overnight. Diethyl ether (4 mL) and the reaction mixture were extracted with CH₂Cl₂ (2 x 20 mL). The organic layers were concentrated to a clear oil that was purified by silica gel chromatography eluting with ethyl acetate (50%, v/v) in hexane. This yielded the desired 6-(2-hydroxyethyl)chroman intermediate as a
15 colorless oil (30 mg, 24 %). ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8'-, 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂, 2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.17, 2.24, 2.28 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.68 (t, J = 6.8 Hz, 2H, 4-CH₂), 3.01 (t, J = 7.5 Hz, 2H, Ar-CH₂), 3.74 (t, J = 7.5 Hz, 2H, OCH₂); ¹³C-NMR
20 (CDCl₃, ppm): 12.0, 15.1, 16.0 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂),

28.0 (CH), 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 62.2 (OCH₂), 72.6 (2-C), 116.8, 122.3, 124.9, 132.4, 133.9 (aryl C), 150.1 (aryl C-O); HRMS (CI, m/z): 458.412154 (M + H⁺, Calc. for C₃₁H₅₄O₂ 458.412384).

5 A solution of pyridinium chlorochromate (32 mg, 0.1 mmol) in anhydrous CH₂Cl₂ (0.5 mL) was treated with a solution of the 6-(2-hydroxyethyl)chroman intermediate (32 mg, 0.07 mmol) in CH₂Cl₂ (0.5 mL). The reaction was stirred for 2 h at which time no starting material was visible by thin layer chromatography. Diethyl
10 ether (2 mL) was added and the resulting solution was filtered through a thin pad of celite. The filtrate as concentrated and yielded a yellow oil (20 mg). This oil was dissolved in t-BuOH (0.5 mL) and treated with phosphate buffer (0.5 mL, 1 N, pH = 4.0), 2-methyl-2-butene (0.1 mL) and NaClO₂ (5.4 mg, 0.05 mmol). After stirring for 40 min, the
15 reaction mixture was extracted with CHCl₃ (6 x 10 mL) and the combined organic layers were dried with Na₂SO₄. The CHCl₃ solution was concentrated to a yellow oil that was purified by preparative thin layer chromatography eluting with ethyl acetate (30%, v/v) and acetic acid (1%) in hexanes. This yielded 22 as colorless oil (20 mg, 63%).
20 ¹H-NMR (CDCl₃/TMS, ppm): 0.87 (m, 12H, 4a'-, 8a'-, 12a'-, 13'-CH₃), 1.0 - 1.6 (m, 24H, 4'-, 8', 12'-CH, 1'-,2'-,3'-,5'-,6'-,7'-,9'-,10'-,11'-CH₂,

2a-CH₃), 1.81 (m, 2H, 3-CH₂), 2.17, 2.24, 2.28 (3 x s, 9H, 5a-, 7a-, 8a-CH₃), 2.66 (t, J = 6.8 Hz, 2H, 4-CH₂), 3.71 (s, 2H, CH₂COOH); ¹³C-NMR (CDCl₃, ppm): 12.0, 15.3, 16.2 (5a-, 7a-, 8a-CH₃), 19.6, 19.7 (CH₃), 20.6, 21.0 (CH₂), 22.6, 22.7 (CH₃), 23.8 (2a-CH₃), 24.4, 24.8 (CH₂), 28.0 (CH), 28.9, 31.2 (3-CH₂), 32.7, 32.8 (CH), 37.3, 37.4, 37.5, 39.4, 40.0 (CH₂), 72.6 (2-C), 117.1, 122.2, 124.9, 132.4, 132.7 (aryl C), 150.2 (aryl C-O), 179.2 (COOH); HRMS (CI, m/z): 472.391583 (M + H⁺, Calc. for C₃₁H₅₂O₃ 472.391644).

10 2,5,7,8-tetramethyl-(2R-(heptyl) chroman-6-yloxy)acetic acid (23)



A solution of hexyltriphenylphosphonium bromide (0.880g, 2.05mmol) in 11.2 ml of anhydrous DME was stirred at room temperature while 0.86 ml (2.06mmol) of 2.4 M n-butyllithium in hexane was added. The resulting red solution was stirred for 2h at room temperature, then a solution of [(+)-S-6-Benzoyloxy-2,5,7,8-tetramethylchroman-2-carbaldehyde (306 mg, 0.944 mmol) in 3 ml of anhydrous DME was added and stirring was continued for 3 h at 65-

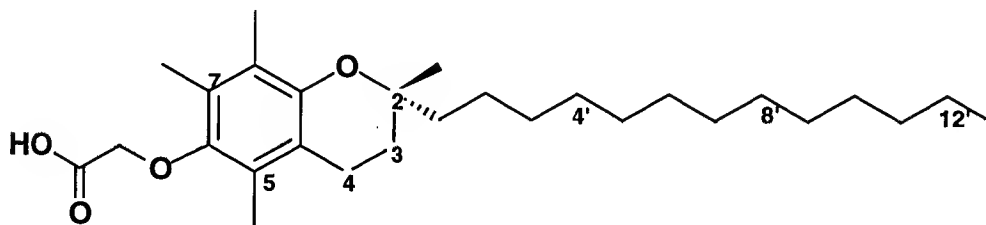
75°C. After cooling, the reaction mixture was poured into cold dilute H₂SO₄ and work up ether was carried out in the usual manner. The ether was concentrated in vacuo to afford the oily material. Product was isolated using column chromatography and eluted with chloroform to yield 46% of the product. The mixture of cis and trans alkene was dissolved in 30 ml of ethyl acetate and 50 mg of 5% palladium on carbon was added, and the mixture was shaken under 40 psi of H₂ for 10 hrs and then filtered through Celilte and rinsed well with ethyl acetate. The filtrate was concentrated and purified by silica gel chromatography eluting with EtOAc in hexane (1:9) to give (2R) 2,5,7,8-tetramethyl-2-(heptyl)-6-chromanol (60% yield) ¹H-NMR (CDCl₃/TMS, ppm):0.89 (s, 3H), 1.3-1.5 (m, 15H), 1.89 (m, 2H), 2.2 (s, 3H), 2.08(s, 3H), 2.23 (s, 3H) , and 2.48 (t, J =6.5 Hz, 2H); MS (CI, m/z):305.35 M + H⁺, Calc. for C₂₀H₃₂O₂ 304.4746).

A solution of 2,5,7,8-tetramethyl-2-(heptyl) chromanol (0.353 g, 1.16 mmol) in N,N-dimethylformamide (20 mL) was treated with methyl bromoacetate (3.4 g, 8.3 mmol) and an excess of powdered NaOH (1.2 g, 30 mmol). The resulting yellow slurry was stirred vigorously for 24 h at room temperature. The reaction was acidified with 5 N HCl and extracted with diethyl ether (3 x 30 ml). The combined ether layers were washed with H₂O (3 x 30 ml) and brine

(1 x 30 ml), and then dried with Na₂SO₄. The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with 19% (v/v) EtOAc and 2% acetic acid in hexanes. The resulting liquid was dissolved in diethyl ether (30 ml),
 5 washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and then dried with Na₂SO₄. The resulting solution was concentrated and dried in vacuo for 48 h. This yielded compound 23 in 36% yield. ¹H-NMR (CDCl₃/TMS, ppm): ¹H-NMR (CDCl₃/TMS, ppm): 0.88 (s, 3H), 1.2-1.5 (m, 15H), 1.88 (m, 2H), 2.1 (s, 3H), 2.18(s, 3H), 2.2 (s, 3H) , 2.55 (t, J
 10 =6.5 Hz, 2H) and 4.78 (s, 2H); HRMS (CI, m/z):363.2535 (M + H⁺, Calc. for C₂₂H₃₅O₄ 363.2541).

2,5,7,8-tetramethyl-(2R-(tridecyl) chroman-6-yloxy) acetic acid (24)

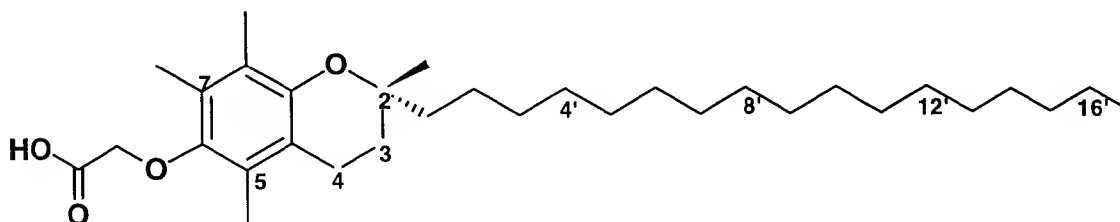
The compounds 24 and 25 were synthesized in manner
 15 identical to the synthesis of 23 using appropriate phosphonium bromide.



$^1\text{H-NMR}$ (CDCl_3/TMS , ppm): 0.83 (s, 3H), 1.25-1.57 (m, 27H), 1.88 (m, 2H), 2.1 (s, 3H), 2.18 (s, 3H), 2.20(s, 3H), 2.55 (t, $J = 6.6$ Hz, 2H) and 4.48 (s, 2H) ; MS (CI, m/z): 447.14 $\text{M} + \text{H}^+$, Calc. For $\text{C}_{28}\text{H}_{46}\text{O}_4$ 446.6732.

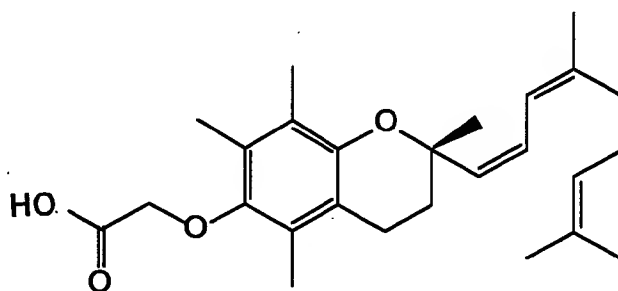
5

2,5,7,8-tetramethyl-(2R-(heptadecyl)chroman-6-yloxy)acetic acid (25)



$^1\text{H-NMR}$ (CDCl_3/TMS , ppm): 0.86 (s, 3H), 1.15-1.67 (m, 35H), 1.88 (m, 2H), 2.16 (s, 3H), 2.20 (s, 3H), 2.23(s, 3H), 2.55 (t, $J = 6.4$ Hz, 2H) and 4.78 (s, 2H) ; MS (CI, m/z): 503.45 $\text{M} + \text{H}^+$, Calc. For $\text{C}_{32}\text{H}_{54}\text{O}_4$ 502.781.

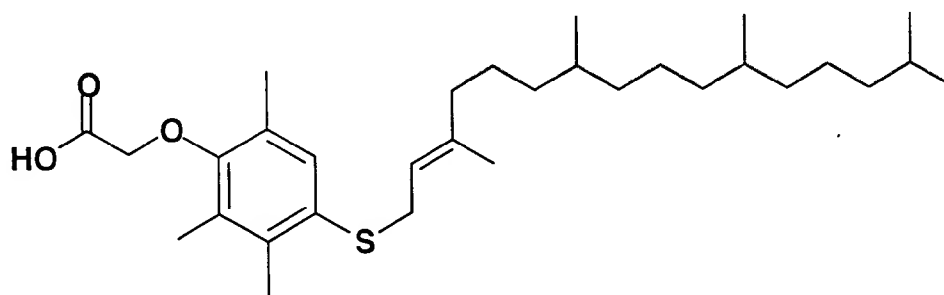
2,5,7,8-tetramethyl-2R-(4,8,-dimethyl-1,3,7 E:Z nonotrien)chroman-6-yloxy) acetic acid (26)



68

Compound **26** was synthesized in a manner identical to the synthesis of compound **18** using nerol instead of pseudoionol. ¹H-NMR (CDCl₃/TMS, ppm) : 1.24 (s, 3H, 2aCH₃), 1.63(m, 1H), 1.68 (s,3H), 1.74(s,6H), 1.92(m, 6H), 2.18 (s,3H), 2.29 (S, 6H), 2.43 (t, J = 6.6Hz, 2H, 4CH₂), 4.68 (s, 2H, OCH₂), 5.64(m, 2H) and 5.27 (m, 1H) ; MS (CI, m/z): 413.24 M +H⁺, Calc. for C₂₆H₃₆O₄ 412.0115.

E.Z, RS, RS, RS-(phytyltrimethylbenzenethiol-6-yloxy) acetic acid (27)



2,3,6-trimethylphenol (1.6 g, 11.8 mmol) was dissolved in 50 mL of anhydrous methanol which had been deoxygenated by bubbling with nitrogen. Ammonium thiocyanate (2.2 g, 28.9 mmol) was added to this solution which was then cooled to 0 °C and bubbled with chlorine gas. The initially colorless homogeneous solution becomes pink and then green with the formation of a white precipitate. The solution was stirred for 1 h at 0 °C and then for a further hour at 20 °C. The dissolved chlorine was removed by bubbling with nitrogen

and the precipitate removed by filtration. Evaporation of the filtrate under reduced pressure followed by drying under high vacuum (0.1 torr) yielded 2.20g (97%) of 2,3,5-Trimethyl-4-hydroxyphenylthiocyanate in a form pure enough for the next step in the synthesis. An analytical sample was recrystallized from hexanes: white crystals, mp 100.3 ° C. ¹H NMR (CDCl₃) δ 7.2 (s, 1 H), 5.0 (s, 1 H) 2.4 (s, 3 H), 2.2 (s, 6 H).

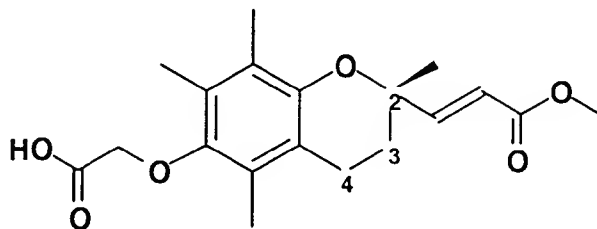
2,3,5-Trimethyl-4-hydroxyphenylthiocyanate (2 g, 10.35 mmol) was dissolved in 100 mL of anhydrous ether containing 25 mL of anhydrous tetrahydrofuran. This solution was added dropwise over 1 h to 100 mL of anhydrous ether containing LiAlH₄ (0.9 g, 24 mmol) at room temperature. After a further hour at 20 ° C, the unreacted LiAlH₄ was destroyed by cooling the heterogeneous mixture to 0 °C and adding moist ether (50 mL), H₂O (50 mL), and 1 N HCl (50 mL). A further 50 mL of water was added and the organic phase was separated and washed with water (2 x 50 mL), NaHCO₃ solution (2 x 50 mL), water (2 x 50 mL), and saturated NaCl (50 mL). The organic phase was dried over anhydrous MgSO₄ and filtered and the solvent removed under reduced pressure. Silica gel column chromatography with 5% ethyl acetate in hexane gave 1.8 g (90%) of 2,3,5-trimethyl-4-hydroxybenzenethiol as a white powder, mp 86 ° C [Lit.¹ mp 86 ° C].

Solution of 2,3,5-trimethyl-4-hydroxybenzenethiol (3 g, 17.83 mmol), isophytol (4.8 g, 16.19 mmol), anhydrous zinc chloride (1.2 g, 8.8 mmol) and 0.2 mL of glacial acetic acid in 30 mL of absolute ether was refluxed for 1 h. The solvent was then removed in vacuo at 50 ° C and the red oil obtained was dissolved in a mixture of 50 mL of petroleum ether and 20 mL of 70% aqueous methanol. The ether layer was dried (Na₂SO₄) and evaporated in vacuo to give a red oil, which was purified by silica gel chromatography eluting with hexane:ether (9:1) to give 3g(38%) E,Z, RS, RS-Phytyltrimethylhydroxybenzenethiol as yellow oil. ¹H NMR (CDCl₃) δ 7.11 (s, 1 H, Ar-H), 5.23 (t, 1 H, vinylic-H), 4.62 (s, 1 H, OH), 3.34 (d, 2 H, Ar-S-CH₂-), 2.41 (s, 3 H, Ar-CH₃), 2.19 (s, 3 H, Ar-CH₃), 2.18 (s, 3 H, Ar-CH₃), 0.83-1.92 (m, 39 H, Phytol chain).

A solution of phytyltrimethylhydroxybenzenethiol (3g, 6.7 mmol) in N, N-dimethyl-formamide (80 mL) was treated with methyl bromoacetate (7.4 g, 48.3 mmol) and an excess of powdered NaOH (7 g, 175 mmol). The resulting pink oil was stirred at RT for 24 h. The reaction mixture was acidified with 5 N HCl and extracted with ether (3 x 150 mL). The combined ether layers were washed with H₂O (3 x 150 mL) and brine (1 x 150 mL), and then dried (Na₂SO₄). The ether solution was concentrated to a yellow oil that was purified by silica gel

chromatography eluting with 20% EtOAc in hexane to give 3 g (88%) of E,Z, RS, RS, RS- (phytyltrimethylbenzenethiol-6-yloxy)acetic acid as a yellow oil. ¹H NMR (CDCl₃) δ 10.90 (s, 1 H, COOH), 8.08 (s, 1 H, Ar-H), 5.30 (t, 1 H, vinylic-H), 4.35 (s, 2 H, CH₂COOH), 3.42 (d, 2 H, Ar-S-CH₂-), 2.34 (s, 3 H, Ar-CH₃), 2.25 (s, 3 H, Ar-CH₃), 2.22 (s, 3 H, Ar-CH₃), 0.83-1.94 (m, 39 H, Phytyl chain). HRMS (CI, m/z): 504.362821(M+H⁺, Calc. for C₃₁H₅₃O₃S 504.363718).

(R)-2[(2,5,7,8-tetramethyl-2-(3 propene methyl ester)chroman-6-yloxy]acetic acid (28)



15

To a slurry of (carbomethoxymethyl)triphenyl phosphonium bromide (1.8 gm, 4.32 mmol) in 12 ml of THF at °C was added 1.66 ml of n-BuLi (2.5M in hexane) dropwise. The resulting solution was removed to room temperature for 2h, and then a solution of (+)S-6-[dimethyl(1,1-dimethylethyl)silyl]-2,5,7,8-tetramethyl chroman-2-carbaldhyde (1.31g, 3.76 mmol) in 7 ml THF was added via

20

cannula. The solution was stirred at room temperature for 44hr and then 10 ml of 1N aq. HCl was added. The layer were separated and then aq. phase was extracted with ether (3 X 15 ml). The combined organic layer were washed with brine, dried over Na₂SO₄ and filtered.

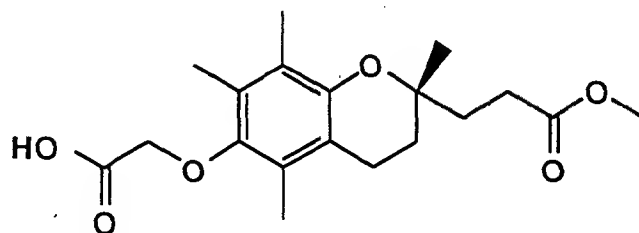
5 After concentration of the filtrate, the crude alkene was purified by flash chromatography eluting with dichloromethane to give mixture of the cis and trans alkene in 93% yield. The silyl ether mixture of cis and trans alkene (3.76mmol) was dissolved in THF and tetra-n-butylammoniumfluoride (0.041mole) was added. After being stirred at
10 23°C for 1.5h, the mixture was poured into water and extracted into ether. The ether extract was dried concentrated and purified by silica gel chromatography eluting with EtOAc in hexane (3:7) and both the cis and trans isomer of 2,5,7,8-tetramethyl-2R-(3'propenemethyl ester)-6-chromanol were isolated and characterized (68% yield) ¹H-NMR
15 (CDCl₃/TMS, ppm): 1.65 (s, 3H, 2a CH₃), 2.12 (m, 2H, 3CH₂), 2.39 (s, 9H, CH₃), 2.48 (m, 2H, 4 CH₂), 3.78 (s, 3H, OCH₃), 6.11 (d, 1H, CH=) and 7.13 (d, 1H, CH=).

A solution of 2,5,7,8-tetramethyl-2R-(3'propene methyl ester)6-chromanol (0.353 g, 1.16 mmol) in N,N-dimethylformamide
20 (20 mL) was treated with methyl bromoacetate (3.4 g, 8.3 mmol) and an excess of powdered NaOH (1.2 g, 30 mmol). The resulting yellow

slurry was stirred vigorously for 24 h at room temperature. The
 reaction was acidified with 5 N HCl and extracted with diethyl ether (3
 x 30 ml). The combined ether layers were washed with H₂O (3 x 30
 ml) and brine (1 x 30 ml), and then dried with Na₂SO₄. The ether
 5 solution was concentrated to a yellow oil that was purified by silica gel
 chromatography eluting with 19% (v/v) EtOAc and 2% acetic acid in
 hexanes. The resulting liquid was dissolved in diethyl ether (30 ml),
 washed with H₂O (3 x 20 mL) and brine (1 x 20 mL), and then dried
 with Na₂SO₄. The resulting solution was concentrated and dried in
 10 vacuo for 48 h. This yielded compound 28 in 40% yield. ¹H-NMR
 (CDCl₃/TMS, ppm): 1.68 (s, 3H, 2a CH₃), 2.11 (m, 2H, 3CH₂), 2.36 (s,
 9H, CH₃), 2.56 (m, 2H, 4 CH₂), 3.70 (s, 3H, OCH₃), 4.78 (s, 2H, OCH₂),
 6.03 (d, 1H, CH=) and 7.03 (d, 1H, CH=); MS (CI, m/z): 337.24 M+H⁺,
 Calc. for C₁₈H₂₄O₆ 336.3867 .

15

2,5,7,8-tetramethyl-(2R-(propionate) chroman-6-yloxy) acetic acid
 (29)



20

The mixture of cis and trans alkene 2,5,7,8-tetramethyl-2R-

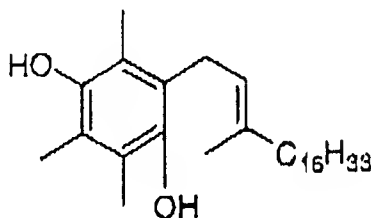
(3 propene methylester)-6-chromanol was dissolved in 30 ml of ethyl acetate and 50 mg of 5% palladium on carbon was added, and the mixture was shaken under 40 psi of H₂ for 24 hrs and then filtered through Celilte and rinsed well with ethyl acetate. The filtrate was concentrated and purified by silica gel chromatography eluting with EtOAc in hexane (1:9) to give compound # 29. ¹H-NMR (CDCl₃/TMS, ppm): 1.62 (s, 3H, 2a CH₃), 2.0-2.3 (m, 6H, CH₂), 2.41 (s, 9H, CH₃), 2.53 (m, 2H, 4 CH₂), 3.67 (s, 3H, OCH₃) and 4.88 (s, 2H, OCH₂); MS (CI, m/z):339.34 M+H⁺, Calc. for C₁₈H₂₆O₆ 338.4025.

10

EXAMPLE 3

Synthesis of all-racemic 1-aza- α -tocopherol analogs:

15 Phytyltrimethylhydroquinone (31)

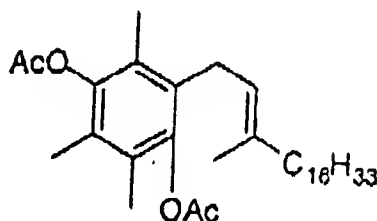


20

The solution of trimethylhydroquinone (30, 7.5 g, 49.28

mmol), isophytol (12 g, 40.47 mmol), anhydrous zinc chloride (3 g, 22.01 mmol) and 0.4 mL of glacial acetic acid in 60 mL of absolute ether was refluxed for 1 h. The solvent was then removed *in vacuo* at 50 ° C and the slurry obtained was dissolved in a mixture of 50 mL of petroleum ether and 20 mL of 70% aqueous methanol. The emulsion formed was destroyed by the addition of 20 mL of ether. The ether layer was dried (Na₂SO₄) and evaporated *in vacuo* to give a red solid paste that was triturated vigorously with 70 mL of petroleum ether. After cooling to -78°C, the suspension was centrifuged and the supernatant petroleum ether was decanted. Cold petroleum ether (10 mL) was added to the crystalline mass, and the operation was twice repeated to give 10 g (47%) of sticky white solid, which was used in the next step without further purification.

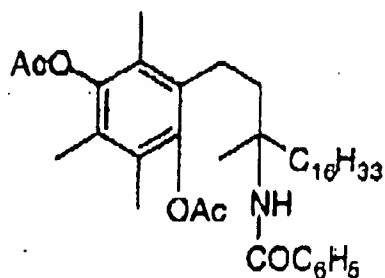
15 Phytyltrimethylhydroquinone diacetate (32)



The crude phytyltrimethylhydroquinone (31, 9 g, 20.1

mmol) was dissolved in dry pyridine (90 g) and acetic anhydride (90 g). After storage for 10 h at room temperature, the mixture was poured onto ice (200 mL) and extracted with ether (2 x 100mL). The ether solution was washed with 3N sulfuric acid (2 x 100 mL), 10% sodium bicarbonate solution (2 x 100mL), and again water (2 x 100 mL), dried (Na_2SO_4), and evaporated to yield 10.2g (95%) of diacetate (32) as a light yellow oil. The oil was used in the next step without further purification.

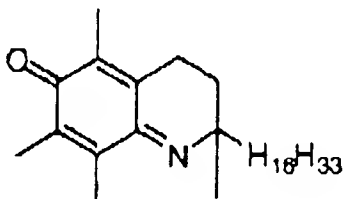
10 Trimethyl(3,7,11,15-tetramethyl-3-benzamidohexadecyl)hydroquinone diacetate (33)



To a mixture of benzonitrile (10 g, 96.97 mmol), concentrated sulfuric acid (10 g) and glacial acetic acid (40 mL) was added, with stirring at 0° C, the diacetate (32, 10 g, 19.43 mmol). The solution was stored for 10 h at room temperature, then poured onto ice (150 mL) and extracted with ether (3 x 100mL). The ether extracts

were washed neutral with water (3 x 100 mL), and saturated sodium bicarbonate solution, dried (Na_2SO_4) and evaporated *in vacuo*. The crude product (21 g) was chromatographed on silica gel eluting with petroleum ether:ether (9:1). The unchanged benzonitrile was first removed. Elution with ether then afforded 11 g (88%) of product which was crystallized from petroleum ether to give analytical sample (33) m.p. 90-92 ° C (lit.1 94-95 ° C).

2,5,7,8-Tetramethyl-2(4,8,12-trimethyltridecyl)3,4-dihydroquinolin-
6(2H)-one (37)



To a solution of the diacetate (33, 13 g, 20.4 mmol) in 60 mL of methanol, solid potassium hydroxide (11 g) was added and the mixture was refluxed under argon for 45 min. After cooling, the solution was diluted with ice-water (100 mL), and extracted under carbon dioxide with ether (3 x 100 mL) yielding 10 g (88%) of crude hydroquinone (34).

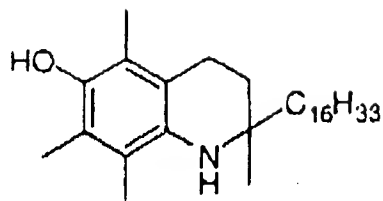
A solution of this product (**34**, 10 g, 18.1 mmol) in absolute ether (110 mL) was added dropwise with stirring during 20 min. to a suspension of lithium aluminum hydride (20 g, 527 mmol) in absolute ether (110 mL). The mixture was refluxed for 14 h and then
5 hydrolyzed under carbon dioxide by the dropwise addition of methanol (10mL) followed by 3N hydrochloric acid (30 mL). After extraction with ether (3 x 200 mL), the crude solid benzylamino derivative (**35**, 8.4 g, 86%) was obtained.

This material (**35**, 8.4 g, 15.6 mmol) was dissolved in glacial
10 acetic acid (120 mL) and hydrogenated at 50 ° C and atmospheric pressure in the presence of 2 g of palladium on charcoal (5%) at room temperature for 22 h (or until the hydrogen absorption ceased). After cooling, the catalyst was filtered off, and the solution was diluted with water (50 mL), extracted with ether (3 x 80 mL) and dried (Na₂SO₄).

15 To this dry and neutral ether solution of **36** was added fresh silver oxide (2.1 g, 8.93 mmol), and the suspension was stirred for 14 h. After filtration under argon and evaporation, 4 g of the crude iminoquinone (**37**) was obtained, which was chromatographed on silica gel eluting with hexanes:ether (200:1) to yield 2.4 g (63%) of
20 pure yellow oil. HMRS (cl, m/z): 428.389883 (M + H⁺, Calc. For C₂₉H₄₉NO 428.389241).

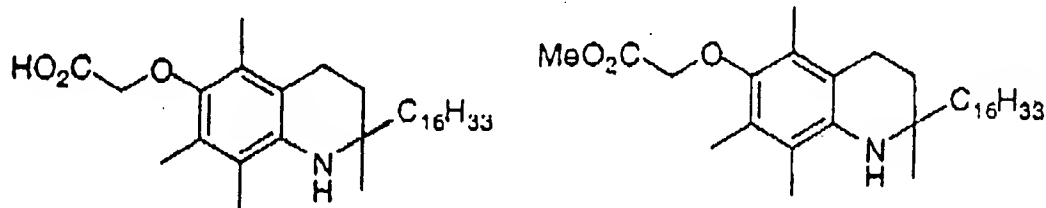
1-Aza- α -tocopherol (38)

5



The iminoquinone (37, 1.34 g, 3.133 mmol) in 10 mL of ether was hydrogenated at room temperature and atmospheric pressure in the presence of 190 mg of Lindlar's catalyst. After 20 h, the catalyst was filtered off under argon and the filtrate was evaporated *in vacuo* to give 810 mg (60%) of crude red oil, which was used in the next step without further purification.

15 1-Aza- α -tocopherol-6-yloxy-acetic acid (39) and 1-Aza- α -tocopherol-6-yloxy-methyl acetate (40)



20

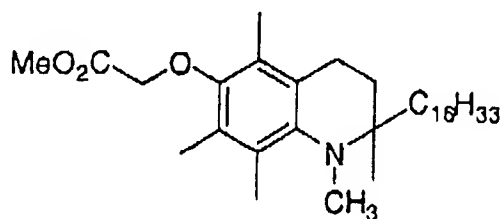
A solution of 1-Aza- α -tocopherol (38, 800 mg, 1.86 mmol)

in DMF (30 mL) was treated with methyl bromoacetate (2.1 g, 13.4 mmol) and an excess of powdered NaOH (1.9 g, 48.4 mmol). The resulting yellow suspension was stirred vigorously for 24 h at room temperature. The reaction mixture was acidified with 5N HCl to pH6, and extracted with diethyl ether (3 x 30 mL). The combined ether layers were washed with water (3 x 40 mL), brine (1 x 40 mL), and then dried (Na₂SO₄). The ether solution was concentrated to a yellow oil that was purified by silica gel chromatography eluting with CH₂Cl₂:MeOH:Hac (97:2:1) to give 300 mg (33%) of sticky yellow solid (39). ¹H-NMR (CDCl₃, ppm) 4.30 (s, 2H, OCH₂), 3.30 (s, 1H, NH), 2.59 (t, 2H, Ar-CH₂), 2.18 (s, 3H, Ar-CH₃), 2.12 (s, 3H, Ar-CH₃), 2.00 (s, 3H, Ar-CH₃), 1.80-0.84 (m, 38H, Ar-CH₂CH₂, NCCH₃ and phytyl chain); ¹³C-NMR (CDCl₃, ppm): 12.01, 12.77, 12.96, 19.60, 19.67, 19.72, 21.09, 22.60, 22.71, 24.42, 24.79, 26.56, 27.95, 32.11, 32.74, 37.26, 37.37, 37.42, 37.55, 37.65 39.34, 41.36, 50.92, 69.50 (aliphitic C), 117.69, 118.55, 125.99, 126.39, 138.07, 146.05 (Ar-C), 173.18 (COOH); (HRMS (CI,m/z): 488.411055 (M + H⁺, Calc for C₃₁H₅₃NO₃ 488.410370) and 150 mg (17%) of yellow oil (40). ¹H-NMR (CDCl₃/TMS, ppm) 4.31 (s, 2H, OCH₂), 3.85 (s, 3H, CO₂CH₃), 3.32 (s, 1H, NH), 2.61 (t, 2H, Ar-CH₂), 2.23 (s, 3H, Ar-CH₃), 2.17 (s, 3H, Ar-CH₃), 2.01 (s, 3H, Ar-CH₃), 1.81-0.86 (m, 38H, Ar-CH₂CH₂, NCCH₃ and

phytyl chain); ^{13}C -NMR (CDCl_3 , ppm) 11.96, 12.67, 12.90, 19.57, 19.63, 19.69, 21.06, 21.95, 22.57, 22.68, 24.38, 24.74, 26.49, 27.91, 32.25, 32.67, 32.70, 37.21, 37.33, 37.53, 39.31, 41.68, 50.43, 51.87, 69.97, (aliphitic C), 116.98, 117.72, 126.01, 126.36, 138.43, 146.18 (Aryl C),
5 169.83 (C=O); **HRMS** (CI, m/z): 502.425330 (M + H⁺, Calc. for C₃₂H₅₅NO₃ 502.426020).

1-Aza-N-methyl- α -tocopherol-6-yloxy-methyl acetate (41)

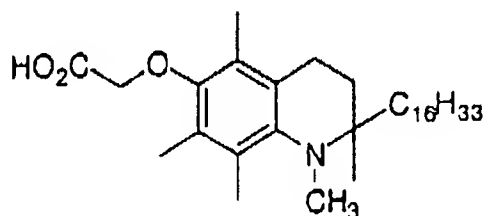
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Following the literature procedure 2, a mixture of powdered
15 KOH (500 mg, 7.77 mmol) in DMSO (30 mL) was stirred at 0°C for 5
min. The methyl acetate (40, 1.3 g, 23.32 mmol) was added, followed
immediately by the addition of CH₃I (3.3 g, 23.32 mmol). Stirring was
continued for 0.5 h (0°C) after which the mixture was poured into
water. Extracts (CH₂Cl₂, 3 x 40 mL) of the final mixture were
20 combined, washed with water (40 mL), brine (40 mL), dried (MgSO₄)
and then evaporated. The resulting yellow oil was chromatographed

over silica gel eluting with 5% ETOAC in hexane to give 810 mg (60%) of light yellow oil. ¹H-NMR (CDCl₃/TMS, ppm) 4.34 (s, 2H, OCH₃), 3.84 (s, 3H, CO₂CH₃), 2.55 (t, 2H, Ar-CH₂), 2.49 (s, 3H, NCH₃), 2.24 (s, 3H, Ar-CH₃), 2.20 (s, 3H, Ar-CH₃), 2.13 (s, 3H, Ar-CH₃), 1.82-0.82 (m, 38H, Ar-CH₂CH₂, NCCH₃ and phytyl chain); ¹³C-NMR(CDCl₃, ppm) 11.72, 13.12, 14.27, 19.55, 19.68, 20.93, 22.57, 22.66, 22.88, 24.35, 24.74, 25.33, 25.37, 27.90, 32.59, 32.71, 37.23, 37.33, 37.51, 37.63, 38.49, 39.06, 39.29, 51.14, 69.57 (aliphitic C), 125.34, 126.36, 127.42, 130.32, 143.89, 150.33 (Ar-C), 169.73 (C=O). The compound **41** was used in the next step without further purification.

1-Aza-N-methyl- α -tocopherol-6-yloxy- acetic acid (**42**)

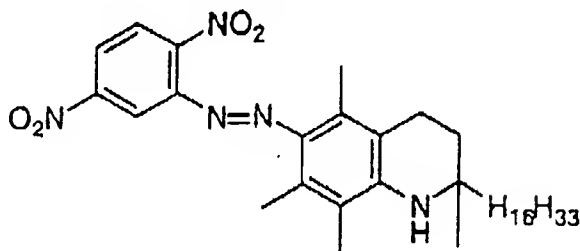


15

To the mixture of the ester (**41**, 740 mg, 14.34 mmol) in ethanol (34 mL) was added dropwise 2N NaOH (9 mL, 10eq. 14.34 mmol). After stirring the light yellow solution at room temperature for 4h, the reaction mixture was acidified with 2N HCl to pH6, and then

extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with water (2 x 100 mL), brine (1 x 100 mL), dried (MgSO₄), and evaporated to give a light yellow oil. The oil was chromatographed over silica gel eluting with 1% HOAc, 30% EtOAc in hexans to give 650 mg (90%) of the product (**42**) as a colorless oil. ¹H-NMR (CDCl₃/TMS, ppm) 10.43 (s, 1H, CO₂H), 4.37 (s, 1H, OCH₂), 2.51 (t, 2H, Ar-CH₂), 2.43 (s, 3H, NCH₃), 2.22 (Ar-CH₃), 2.12 (s, 3H, Ar-CH₃), 1.81-0.80 (m, 38H, Ar-CH₂CH₂, NCCH₃ and phytyl chain); ¹³C-NMR (CDCl₃, ppm): 11.77, 13.18, 14.28, 19.68, 19.73, 20.80, 20.96, 22.62, 22.72, 23.00, 24.00, 24.41, 24.80, 25.37, 27.80, 32.77, 37.28, 37.30, 37.74, 38.00, 39.00, 39.35, 54.69, 69.17 (aliphitic C), 125.11, 126.55, 127.20, 130.63, 142.00, 149.60 (Ar-C), 173.62 (COOH); HRMS (CI, m/z): 502.426327 (M + H⁺, Calc. For C₃₂H₅₅NO₃ 502.426020).

6-(2,4-Dinitrophenylazo(2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-1,2,3,4-tetrahydroquinoline (43)



2,4-dinitrophenylhydrazine (1.95 g, 9.82 mmol) was

5 dissolved in 34 mL of hot absolute ethanol and 3.4 mL of concentrated
sulfuric acid was carefully added. The hot red solution was cooled to
room temperature and the iminoquinone (37, 1.00 g, 2.34 mmol) in 12
mL of absolute ethanol was added with stirring. The mixture was
10 allowed to stand at room temperature for 70 h, then diluted with 120
mL of water and extracted with ether (3 x 100 mL). The combined
ether extracts were washed neutral with water (6 x 100 mL), dried
(NaSO₄), and evaporated *in vacuo* to give 2.9 g of a partly crystalline
dark violet mass. This product was chromatographed over silica gel
10 eluting with 5% ether in petroleum ether to yield 1.35 g (95%) of violet
crystals (**43**), m.p. 72-73 °C (lit.¹ 73-75). **HRMS** (CI, m/z):
608.417199 (M + H⁺, Calc. For C₃₅H₅₃N₅O₄ 608.417581).

15 **EXAMPLE 4**

Cell Culture Conditions

All test cell lines were cultured at 37°C in 5% CO₂ in
standard media supplemented with fetal calf serum, using established
20 standard conditions. Plastic adherent cells were disassociated with
trypsin, washed, counted, and used directly in experiments. All cells

were examined routinely to verify no mycoplasma contamination.

EXAMPLE 5

5

Solubility and Dilution of Novel Tocopherol and Tocotrienol Compounds

All compounds were handled as if they were light sensitive (photodegradable). All compounds were initially dissolved in absolute
10 ethanol and subsequently diluted to a final concentration of 0.5% ethanol with the appropriate media.

EXAMPLE 6

15

Determination of Effective Concentration (EC₅₀) to Induce Apoptosis

Whereas the parental non-structurally modified forms of tocopherols do not exhibit effective apoptotic properties against a battery of tumor cells, fifteen out of twenty-nine RRR- α -tocopherol
20 compounds and two out of five 1-aza- α -tocopherol analogs, structurally modified via ether linked moieties of different composition

and size were extremely effective at inducing tumor cells to undergo apoptosis while having no apoptotic inducing properties on normal cells. Compounds 1, 2, 3, 7, 8, 9, 12, 15, 17, 19, 20, 21, 22, 25, 26, 27, 39, and 42 exhibit effective growth inhibitory (apoptotic inducing) properties specific for human cancer cells from a wide variety of cell lineages, including (i) breast (estrogen responsive Michigan Cancer Foundation human breast cancer cell line number 7, MCF-7 McGuire; non-estrogen responsive M.D. Anderson metastatic breast human cancer cell line, MDA-MB-435; and, estrogen non-responsive M.D. Anderson metastatic human breast cancer cell line, MDA-MB-231); (ii) prostate (androgen responsive human prostate cancer cell line, LnCaP and the androgen non-responsive human prostate cancer cell line, PC-3 and the DU-145 cell line); (iii) promyelocytic leukemia cells (human Promyelocytic Leukemia Cell Line, HL-60), lymphoid cell lines Jurkat and HL-60; (iv) cervical (human cervical cancer cell line, ME-180); (v) ovarian (human ovarian cancer cell line, C-170 cells); (vi) endometrial (human endometrial cancer cell line, RL-95-2 cells); (vii) colon cell lines DLD-1; and (viii) lung cell line A-549. Normal primary breast cells (normal primary early passage human mammary epithelial cells, HMEC) and immortalized, non-tumorigenic mammary cells (Michigan Cancer Foundation immortalized but non-tumorigenic human

mammary number 10A cells, MCF-10A) do not undergo apoptosis when cultured with the above pharmacodynamically designed forms of tocopherol.

The effective therapeutic dose of novel reagents for
5 controlling cancer growth is referred to as the growth inhibitory
concentration (IC_{50}) or effective concentration (EC_{50}) that blocks 50%
cancer growth via DNA synthesis inhibition, cell cycle blockage and/or
cell death. The apoptotic EC_{50} for a battery of test cancer cells for the
twenty-nine novel RRR- α -tocopherol compounds and two of the five 1-
10 aza- α -tocopherol analogues of this invention are presented in Tables 1
and 2.

TABLE 1

Apoptosis Induced by Novel Tocopherol Compounds (EC_{50} range $\mu\text{g/ml}$)

Cell Type	VES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<u>Breast Cancer</u>																
HMEC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NT
MCF-10A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NT
MDA-MB-435	5-10	5-10	10-20	5-10	N	N	N	5-10	5-10	5-10	N	N	5-10	N	N	20-30
MDA-MB-231	5-10	5-10	10-20	5-10	N	N	N	5-10	5-10	5-10	N	N	5-10	N	N	20-30
MCF-7	10-20	5-10	20-30	20-30	N	N	N	10-20	10-20	10-20	N	N	5-10	N	N	20-30
T47D	N	N	N	N	N	N	N	N	NT	NT	NT	NT	NT	NT	NT	NT
<u>Cervical</u>																
ME-180	10-20	1-5	5-10	10-20	N	N	N	5-10	5-10	5-10	N	N	5-10	N	N	N
<u>Ovarian</u>																
C-170	N	10-20	10-20	10-20	N	N	N	10-20	10-20	N	N	N	10-20	N	N	N
<u>Endometrial</u>																
RL-95-2	10-20	10-20	10-20	10-20	N	N	N	5-10	1-5	5-10	N	N	5-10	N	N	N
<u>Prostate</u>																
PREC	N	N	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
LnCaP	5-10	5-10	5-10	5-10	N	N	N	2.5-5	5-10	5-10	N	N	>20-30	NT	N	NT
PC-3	10-20	5-10	5-10	5-10	N	N	N	5-10	5-10	5-10	NT	N	10-20	N	N	NT
DU-145	10-20	5-10	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
<u>Colon</u>																
HT-29	5-10	10-20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
DLD-1	10-20	10-20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
<u>Lung</u>																
A-549	20-30	10-20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
<u>Lymphoid Cells</u>																
Myeloma	10-20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Raji	10-20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Ramos	10-20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Jurkat	10-20	10-20	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
HL-60	10-20	5-10	10-20	10-20	N	N	N	5-10	10-20	10-20	N	N	20-30	N	N	NT

EC_{50} 50 $\mu\text{g/ml}$ of tocopherol compounds 1-29 inducing 50% apoptosis; N = No apoptosis when treated for 2 days with 1-60 EC_{50} $\mu\text{g/ml}$ of tocopherol compounds 1-29; NT = Not tested; * = compounds exhibiting toxicity.

TABLE 2

Apoptosis Induced by Novel Tocopherol Compounds (EC_{50} range $\mu\text{g/ml}$)

Cell Type	16	17	18	19	20	21	22	23	24	25	26	27	28	29	39	42	43
<u>Breast Cancer</u>																	
HMEC	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
MCF-10A	NT	N	NT	N	N	N	NT	N	NT	N	N	NT	NT	NT	NT	NT	NT
MDA-MB-435	N	NT	N	10-20	10-20	N	NT	N	N	N	20-40	NT	NT	NT	10-20	10-20	PPT.
MDA-MB-231	N	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
MCF-7	N	10-20	N	10-20	5-10	N	15-20	N	N	N	N	10-20	NT	NT	NT	NT	NT
T47D	NT	10-20	NT	N	5-10	NT	NT	NT	NT	NT	N	NT	NT	NT	NT	NT	NT
<u>Cervical</u>																	
ME-180	NT	20-30	N	1-5	1-5	1-5	NT	NT	NT	N	*	NT	NT	NT	NT	NT	NT
<u>Ovarian</u>																	
C-170	NT	20-30	N	1-5	*	N	NT	NT	NT	N	20-30	NT	NT	NT	NT	NT	NT
<u>Endometrial</u>																	
RL-95-2	NT	NT	NT	NT	NT	N	NT	NT	NT	N	20-30	NT	NT	NT	NT	NT	NT
<u>Prostate</u>																	
PREC	NT	NT	N	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Lncap	NT	10-20	NT	5-10	5-10	N	NT	NT	N	N	10-20	10-20	NT	NT	NT	NT	NT
PC-3	NT	NT	NT	N	5-10	N	NT	N	N	20-30	N	NT	NT	NT	NT	NT	NT
DU-145	NT	NT	NT	5-10	5-10	N	NT	N	N	20-30	N	NT	NT	NT	NT	NT	NT
<u>Colon</u>																	
HT-29	NT	N	N	NT	NT	NT	NT	N	NT	N	N	NT	NT	NT	NT	NT	NT
DLD-1	NT	NT	NT	NT	NT	NT	NT	NT	N	N	20-40	NT	NT	NT	NT	NT	NT
<u>Lung</u>																	
A-549	NT	N	N	20-30	20-30	NT	NT	NT	NT	NT	N	NT	NT	NT	NT	NT	NT
<u>Lymphoid Cells</u>																	
Myeloma	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Raji	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Ramos	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Jurkat	NT	10-20	N	10-20	10-20	NT	NT	NT	NT	NT	20-30	NT	NT	NT	NT	NT	NT
HL-60	NT	10-20	N	10-20	10	NT	NT	NT	NT	NT	N	NT	NT	NT	NT	NT	NT

EC_{50} 50 $\mu\text{g/ml}$ of tocopherol compounds 1-29 inducing 50% apoptosis; N = No apoptosis when treated for 2 days with 1-60 EC_{50} $\mu\text{g/ml}$ of tocopherol compounds 1-29; NT = Not tested; * = compounds exhibiting toxicity.

EXAMPLE 7

Bioassay for Apoptosis

Cells were cultured at 1.5×10^5 cells/well in 12 well plates.

5 Cells were allowed to adhere overnight, then incubated with novel test compounds at 0.01, 0.1, 1, 5, 10 & 20 $\mu\text{g/mL}$ for 1, 2 and 3 days. After treatment, cells (floating + trypsin released adherent cells) were pelleted, washed and stained with 2 $\mu\text{g/ml}$ DAPI (4',6-diamidine-2'-phenylindole dihydrochloride) in 100% methanol for 15 minutes at
10 37°C and/or TUNEL stained, then viewed using a Zeiss ICM 405 microscope. Cells whose nucleus contained clearly condensed or fragmented chromatin were scored as apoptotic. Data are presented as percent cells undergoing apoptosis.

15

EXAMPLE 8

Bioassay for DNA synthesis arrest

To assay DNA synthesis, all cells were used at $2.5 \times 10^5/\text{ml}$.

20 Cells were treated with each of the compounds 1-29 (Tables 1 and 2) at concentrations of 0.01, 0.1, 1, 5, 10 and 20 $\mu\text{g/mL}$ and 200 μl of each

treatment group were plated in quadruplicate in a 96 well culture plate (Corning, Corning NY). Experiments were done in duplicate, one plate used for viability testing and the other plate for examination of ^3H -TdR uptake to monitor DNA synthesis. Plates were cultured for 48 hours at 5 37°C, 5% CO₂. Eight hours prior to the end of incubation, ^3H -TdR was added to one of the duplicate plates and incubation continued for 8 hours. The cells were then harvested (trypsinization was required to harvest adherent cells), and isotope uptake was determined as counts per minute (cpm). For viability studies, at the end of the incubation, 10 the cells were removed from the wells and viability checked by the Trypan Blue Exclusion method. Percent viability and percent DNA synthesis in comparison to untreated or vehicle treated cells of each treatment group were calculated.

15

EXAMPLE 9

Bioassay for Cell Cycle Arrest

The cells were cultured with novel test agents for 2-3 days, 20 fixed in 95% ethanol and stained with propidium iodide overnight. DNA content was determined using a Coulter Epics Elite Flow Cytometer

with an argon laser setting of 488 nm. Cell size was measured simultaneously, and data were analyzed as to percent cells in each cell cycle phase using the Coulter Multicycle Program.

5

EXAMPLE 10

Bioassay for Cellular Differentiation

To determine if the novel compounds were inducing
10 cellular differentiation, the cells were cultured on cover slips, fixed in 95% ethanol and stained with a lipid specific stain for detection of milk lipids. Additionally, cells were examined by immunohistology and by Western analyses for presence of milk protein casein, using polyclonal antibodies produced in the lab.

15

EXAMPLE 11

DNA Synthesis Arrest Effects

20 The cells were cultured for 48 hours, pulsed 8 hours with tritiated thymidine, harvested and counted. Data are presented as

counts per minute. Verification of DNA synthesis arrest is determined by reduced tritiated thymidine uptake by cells treated with test compounds. Further verification of DNA synthesis arrest is determined by propidium iodide staining and standard cell cycle analyses.

5

EXAMPLE 12

Mechanisms of Induction of Apoptosis

10 The mechanism of induction of apoptosis by these compounds appears to involve three distinct apoptotic signaling pathways; namely, activation of latent transforming growth factor-beta (TGF- β), activation of the Fas/Fas ligand signaling pathways, and signaling by the stress kinase (c-Jun N-terminal Kinase) pathway.

15 TGF- β s are potent growth inhibitory molecules that are known to inhibit cell growth by inhibition of DNA synthesis arrest and by induction of apoptosis. TGF- β s are involved only in induction of the apoptotic pathway, i.e., there is no evidence currently that the TGF- β s effect DNA synthesis arrest; however, this possibility has not been
20 completely ruled out. TGF- β s are made and secreted by cells in a latent non-active form. To be effective as tumor growth inhibitors, the latent

TGF- β s must be activated by induction of cell surface proteins that provide a proper structure for processing and activating proteases that cut the latent protein and release the active TGF- β .

The compounds of the present invention are shown to
5 activate proteases such as cathepsin D family proteases, and upregulate the mannose-6-phosphate receptor which binds inactive TGF- β and permits activation via proteases. Active TGF- β signals via cell membrane TGF- β receptors I and II to activate down stream kinases referred to as stress kinases or c-Jun N-terminal Kinases (JNK) which
10 phosphorylate and activate transcription factors c-Jun, ATF-2 and Elk-1. Prolonged activation of transcription factor c-Jun causes tumor cells to undergo apoptosis. These transcription factors, acting as homodimers or heterodimers with a multitude of transcription factor partners activate proapoptotic genes and/or downregulate
15 antiapoptotic genes leading to DNA fragmentation. The compounds of the present invention do not generate an anti-proliferative outcome to TGF- β signaling in normal non-tumor cells.

A second apoptotic inducing mechanism called the Fas/Fas ligand apoptotic signaling pathway is activated by the novel compounds
20 of the present invention. Activated Fas/Fas ligand signaling may lead to rapid cell death by apoptosis. Thus, for tumor cells to escape death by

Fas/Fas ligand, they must inactivate this most important apoptotic pathway. The mechanism for inactivation of the Fas/Fas ligand signaling pathway by tumor cells varies; however, many tumor cells down regulate the expression of Fas receptor and Fas ligand on their
5 membranes.

Most important, R,R,R-2-(2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxy)acetic acid (**1**) has been shown to induce Fas/Fas ligand resistant tumor cells to become Fas/Fas ligand sensitive. Compound **1** also has the ability to enhance the expression
10 of Fas ligand on the membrane of LNCaP prostate cells. Studies show that Fas signaling resistant human breast cancer cells retain the Fas receptor in their cytoplasm, but when cultured with compound **1**, the Fas receptor is transported from the cytoplasm to the membrane; thereby rendering the cells Fas signaling sensitive. Furthermore, this
15 compound is synergistic in anti-Fas triggered apoptosis in that greater amounts of cell killing is obtained with both human breast and prostate cancer cells when co-treated versus when treated separately. The ability of compound **1** to convert Fas signaling resistant tumor cells to Fas signaling sensitive tumor cells and to exhibit synergistic killing
20 effects provides an extremely important mechanism for destruction of tumor cells both by the host immune surveillance system as well as by

pharmaceutical intervention. The compounds of the present invention do not activate the Fas signaling pathway of normal non-tumor cells.

These compounds activate the JNK kinase signaling pathway, perhaps by TGF- β and Fas/Fas ligand signaling. Prolonged
5 activation of JNK results in prolonged activation of c-Jun and ATF-2 transcription factors, which are postulated to play a role in expression or repression of proapoptotic and antiapoptotic genes, respectively.

10 **EXAMPLE 13**

Mechanism of Induction of DNA Synthesis Arrest, Cell Cycle Arrest and Cellular Differentiation

The mechanisms of growth inhibition by DNA synthesis
15 arrest, cell cycle arrest and by induction of cellular differentiation have not been characterized as fully as the mechanism of growth inhibition by apoptosis. Studies show that the compounds of the present invention have profound effects on the cell cycle, inducing DNA
synthesis arrest of approximately 95% of the tumor cells within 24
20 hours of treatment. Tumor cells cultured with the compounds disclosed herein are growth inhibited in the G1 cell cycle phase,

undergo morphological changes and express milk lipids, an indication that the cell cycle blocked cells have undergone differentiation. P21, a gene known to be an inhibitor of entrance of cells from the G1 cell cycle phase to the S phase of the cell cycle, and the mRNA, as well as the protein of P21 gene, is up-regulated by treatment of MDA-MB-435 human breast cancer cells with compound 1.

EXAMPLE 14

10

In Vivo Potential for Human Cancer Cells

The present invention has potential for use as therapeutic agents. In vivo studies of tumor growth and metastasis of human tumor cells either ectopically or orthotopically transplanted into immune compromised animals, such as nude mice, or in vivo studies employing well recognized animal models are conducted. Inhibition of growth of human tumor cells transplanted into immune compromised mice provide pre-clinical data for clinical trials. In vivo studies include two human tumor cell models, the metastatic non-estrogen responsive MDA-MB-435 breast cancer model, and the androgen non-responsive PC-3 prostate cancer model.

MDA-MB-435 Breast Cancer Model:

Pathogen free MDA-MB-435 human breast cancer cells stably transfected with a marker protein (fluorescent green protein) are grown as a solid tumor in immune compromised nude or SCID mice. The tumors are removed, and 1 mm sections of equal size are orthotopically transplanted into the mammary fat pad or ectopically transplanted into the hind flank of female nude mice. Tumor growth, metastasis, and death of the animals are determined. Tumor growth is measured by caliper evaluations of tumor size. At the time of sacrifice, tumors are removed, measured for size, and used for histochemical examination. Organs such as spleen, lymph nodes, lungs, and bone marrow, are examined for metastatic MDA-MB-435 cells by histochemical staining of tissue sections for expression of the marker fluorescent green protein.

PC-3 Prostate Cancer Model

Pathogen free PC-3 human prostate cancer cells stably transfected with a marker protein (fluorescent green protein) are grown as a solid tumor in nude mice. The tumors are removed, and 1 mm sections of equal size are ectopically transplanted into the hind flank of male nude mice. Tumor growth, metastasis, and death of the

animals are determined. At the time of sacrifice, tumors are removed, measured for size, and used for histochemical examination. Organs such as spleen, lymph nodes, lungs, bone marrow, are examined for metastatic PC-3 cells by histochemical staining of tissues for expression
5 of the marker fluorescent green protein.

Skin Cancer Animal Model

Skin cancer is induced in SENCAR and SKH-1 hairless mice by ultraviolet irradiation and chemical (DMBA) treatments. In
10 addition, mice specifically expressing the oncogene Her-2/neu in skin basal cells that spontaneously develop skin cancer are used. The compounds disclosed herein are topically applied to the skin daily, before and after skin cancer initiation, and development of skin papilloma formation is assessed. Control mice are treated identically
15 except that they receive vehicle treatments topically applied to their skin. The efficacy of these compounds in treating papilloma's as well as their ability to affect malignant conversion when supplied prior to premalignant progression is monitored.

EXAMPLE 15

Supplementation with Novel Compounds

Prior to initiation of the in vivo experiments, the
5 compounds of this invention that exhibit the greatest amount of tumor
cell killing are administered to nude, SCID, transgene, and other mice at
varying levels to establish the highest level of compound that can be
administered safely without adverse effects. The compounds are
administered in a model-appropriate manner; e.g., orally, injections,
10 including injections directly into the target organ, or topically. After
establishing the highest level of the compounds that can be tolerated
and effective administration routes, the novel compounds are
administered to the mice on a daily basis, and tumor growth and
progression is determined as described above.

15

EXAMPLE 16

Establishing Maximum Tolerated Dose (MTD) of Compound 1

20 Prior to conducting the preclinical chemotherapeutic
studies, compound 1 is assayed in non-tumor Balb/c female mice in an

effort to establish the maximum tolerated dose. Compound 1 is dissolved in 100% ethanol to establish a stock solution (2 grams of compound 1 in 5 mls of 100% ethanol) and diluted in peanut oil (non vitamin E supplemented) to yield 20, 10, and 5 mg/0.1 ml. RRR- α -tocopherol succinate (VES; the succinate moiety is attached to RRR- α -tocopherol via an ester linkage) was solubilized and diluted as described above to yield 20 mg/0.1ml/gavage, serving as an additional control. A total of 30 Balb/c female mice were placed into 6 groups (5 mice/group) and supplemented by gavage with compound 1 daily for a total of 23 days. Group 1 mice were non treated and served as control; group 2 mice were the vehicle control group and received 0.1ml of vehicle (ethanol + peanut oil equivalent to treatment groups)/mouse/day for a total of 23 days, group 3 mice received 20 mg/ml of VES daily for a total of 23 days. Treatment groups 3, 4 and 5 were gavaged daily for 23 days with 5, 10, and 20 mgs of compound 1. Compound 1 at 5, 10, and 20 mg/day, administered daily by gavage to mice in groups 3, 4, and 5, respectively, for 23 days is tolerated well by the mice. Weights are determined weekly and there is no significant difference in the weights of the mice receiving compound 1 at 5, 10, and 20 mg/day when compared with the weights of the VES treated, untreated and vehicle treated control mice (Figure 8, data is for days

11 and 23 days treatment). Mice remained active and showed no signs of toxicity. At the completion of the MTD studies, mice were sacrificed and no evidence of toxicity was observed upon autopsy.

5

EXAMPLE 17

Chemotherapeutic effectiveness of compound 1

The maximum tolerated dose studies show that compound 1 administered at the highest level (20 mg/day/23 days) is not toxic; however, due to the non-toxic levels used, an maximum tolerated dose was not established. For the preclinical chemotherapeutic studies, in the absence of an maximum tolerated dose, the mice are supplemented with 30 and 20 mgs/0.1ml by gavage daily for 21 days. Compound 1 is dissolved in 100% ethanol at 2 grams of compound 1/5mls of ethanol, and diluted in peanut oil (nonsupplemented vitamin E) to yield 30 and 20 mgs of compound 1 per 0.1ml. Vehicle control consisted of a mixture of ethanol and peanut oil equivalents. The preclinical chemotherapeutic studies are conducted with compound 1, utilizing human MDA-MB-435 human breast cancer cells, human DU-145 prostate cancer cells, and human HT-20 colon tumor cells transplanted

into Balb/c immune compromised nude mice.

A total of 40 immune compromised nude mice are utilized for testing each test compound (two treatment groups at 20 and 30 mg/day) in each tumor cell type. Four experimental treatment groups of 10 mice each (vehicle/0.1ml/gavage daily for 21 days, compound 1 at 30 mgs of compound #1/0.1ml gavage/daily for 21 days, and compound 1 at 20 mgs/of compound 1/0.1ml gavage daily for 21 days, for each tumor type are used. An established dosage of effective chemotherapeutic drugs are used in the xenograft studies. Taxol at 20/mg/kg, administered intraperitoneally daily for 5 days, is used for a positive control for human MDA-MB-435 breast cancer xenografts; Mitoxantrone at 1 mg/kg, administered daily intravenously for 5 days, is used as the positive control for human DU-145 prostate cancer cells; and 5 Fluorouracil (5 FU) at 30 mg/kg, administered daily for 5 days, is used for a positive control for human HT-29 colon cancer cells. Tumor cells are transplanted subcutaneously into the left flank and permitted to grow to approximately 70 mg in size, test compound treatments by daily gavage are initiated, and continue for a total of 21 days. Mice are monitored daily for tumor size by caliper measurements. Mice are treated for 21 days; however, the mice are sacrificed when the tumors of the vehicle control reached 500 mg in

weight. At the completion of the protocol, tumors are excised and tumor size established by weight. At the time of sacrifice, serum, tumor, and heart muscle are taken from animals in the two compound 1 test groups in order to establish levels of compound 1 via HPLC analyses (data not included). The chemotherapeutic effectiveness of compound 1 administered daily at 30 and 20 mgs/day is determined by comparing the tumor size of the two treatment groups with the vehicle control group for each of the three tumor types, and by comparing the size of the tumor in the two treatment groups with the tumor group from the positive control. Treatment is for 21 days, experiments are continued until mean weight of tumor for vehicle control reaches 500 mgs.

The *in vivo* preclinical chemotherapeutic data are depicted in Tables 3 and 4 and Figure 9A-C and are presented for 21-22 days of treatment. Treatment of nude mice transplanted with human MDA-MB-435 breast cancer cells with compound 1 for 21 days at 20 and 30 mg/day by gavage reduced the mean tumor weight (mg) of the transplanted tumor cells by 20.7 and 44.6%, respectively when compared to the mean tumor weight of the vehicle control group at day 21 after treatment. The positive control, Taxol, reduces the mean tumor growth, when compared to mean tumor growth of vehicle

control group, by 90.3% (Table 3, 4 & Figure 9A). Data are converted to percent mean tumor growth in relation to weight gain at the beginning of treatment (day 1) and are depicted in Table 4. Treatment of nude mice transplanted with human DU-145 prostate cancer cells with compound 1 for 21 days at 20 and 30 mg/day by gavage reduces the mean tumor weight (mg) of the transplanted tumor cells by 31.5 and 24.8%, respectively, when compared to the mean tumor weight of the vehicle control group at day 22 (Table 3, 4 & Figure 9B). The positive control, mitoxantrone reduces the mean tumor growth, when compared to mean tumor growth of vehicle control group, by 27.9%. Treatment of immune compromised nude mice transplanted with human HT-29 human colon cancer cells with compound 1 for 21 days at 20 and 30 mg/day by gavage reduces the mean tumor weight (mg) of the transplanted tumor cells by 33.3 and 34.5 %, respectively when compared to the mean tumor weight of the vehicle control group at day 22 (Table 3, 4 & Figure 9C). The positive control, 5 Fluorouracil, reduces the mean tumor growth, when compared to mean tumor growth of vehicle control group, by 31.4%.

TABLE 3: MDA-MB-435 Human Breast Cancer Cells Transplanted in to Nude Mice

		Mean tumor Weights (mg) Following Treatments						
Treatments		1	4	7	10	14	17	21
5	Vehicle	70.1	98.2	119.5	153.2	172.8	203.7	226.1
	#1 (20 mg)	70.1	85.8	123.9	127.9	147.2	143.8	179.4
	#1 (30 mg)	68.1	85.7	97.3	98.4	126.6	118.7	125.2
	Taxol	70.7	71.5	38.2	11.7	5.7	3.6	6.8

DU-145 Human Prostate Cancer Cells Transplanted into Nude Mice

		Mean tumor Weights (mg) Following Treatments						
Treatments		1	5	8	12	15	19	22
	Vehicle	66.5	111.4	167.1	258.5	351.1	458.3	540.9
	#1 (20 mg)	66.5	103.5	137.9	208	254.8	362.2	370.5
	#1 (30 mg)	67.8	102.5	131.3	241.2	256.8	379.9	407
15	Mitoxantrone	66.8	107.2	141.1	185.4	205.2	325.3	389.4

HT-29 Human Colon Cancer Cells Transplanted into Nude Mice

		Mean tumor Weights (mg) Following Treatments						
Treatments		1	5	8	12	15	19	22
	Vehicle	80.3	91.7	133.2	185.6	204.8	260.1	372.2
20	#1 (20 mg)	59.5	84.0	97.0	124.6	147.3	198.0	248.2
	#1 (30 mg)	40.0	83.0	96.7	129.3	144.1	176.1	243.8
	5-FU	59.3	87.5	105.8	145.8	141.6	212.3	255.5

Table 4: Percent mean tumor weight gain (mg) following treatments

MDA-MB-435 Human Breast Cancer Cells

Percent Mean tumor Weight Gain (mg) Following
Treatments¹

5	Treatments	1	4	7	10	14	17	21
	Vehicle	100	140.1	170.5	218.5	246.5	290.6	322.5
	#1 (20 mg)	100	122.4	176.7	182.5	210.0	205.1	255.9
	#1 (30 mg)	100	125.8	142.9	144.5	185.9	174.3	178.6
	Taxol	100	101.1	-46.0	-83.7	-91.8	-99.5	-90.3

10 DU-145 Human Prostate Cancer Cells

Percent Mean tumor Weight Gain (mg) Following Treatments

	Treatments	1	5	8	12	15	19	22
	Vehicle	100	167.5	251.3	388.7	528.0	458.3	689.2
	#1 (20 mg)	100	155.6	207.4	312.8	383.2	362.2	544.7
15	#1 (30 mg)	100	151.2	193.7	355.8	378.8	379.9	560.3
	Mitoxantrone	100	160.5	211.2	277.5	307.2	487.0	582.9

HT-29 Human Colon Cancer Cells

Percent Mean tumor Weight Gain (mg) Following Treatments

	Treatments	1	5	8	12	15	19	22
20	Vehicle	100	152.1	220.9	307.8	339.6	431.3	617.2
	#1 (20 mg)	100	141.2	163.0	209.4	247.6	332.8	417.1
	#1 (30 mg)	100	139.5	162.5	217.3	242.2	296.0	409.7
	5-FU	100	147.6	178.4	245.9	247.2	358.0	430.0

25 ¹Percent Mean Tumor Weight Gain (mg) following treatment was determined by dividing the mean tumor weight at various time periods following treatment by the mean tumor weight at day 1 within the same treatment group and multiplying by 100.

EXAMPLE 18

Preparation of Stock Solution, Vehicle and Compound 1 Dilutions

Compound 1 was prepared weekly, and stored at 4°C. The
5 preparation procedures are as follows:

Stock Solution of Compound 1:

Dissolve 2 grams of compound 1 in 5 mls of 100% ethanol
(ETOH) and vortex at 37°C. This is the maximum amount of compound
1 that will go into solution.

10 For the following dilutions, dry compound 1 is added to
yield the appropriate levels of compound 1 while keeping the ethanol
levels equal in the two experimental groups and the vehicle control
group.

Compound 1 at 30 mg/0.1ml gavage/mouse:

15 Combine 1 ml of compound 1 stock solution, 3
mls of vitamin E depleted peanut oil and 800 mg of compound #1 (dry)
and vortex at 37°C.

Compound 1 at 20 mg/0.1ml gavage/mouse:

20 Combine 1 ml of compound 1 stock solution, 3 mls of
vitamin E depleted peanut oil and 400 mg of compound #1 (dry) and
vortex at 37°C approximate 2 h until in solution.

Compound 1 at 10 mg/0.1ml gavage/mouse:

Combine 1 ml of compound 1 stock solution and 3 mls of vitamin E depleted peanut oil.

Vehicle:

5 Combine 1 ml ETOH 3 mls of vitamin E depleted peanut oil.

EXAMPLE 19

10 Chemopreventive properties of compound 1 in an ACI rat cancer model.

Compound 1 is used in vivo to treat transplanted human breast, prostate, and colon tumors transplanted in immune compromised nude mice. The chemopreventive effectiveness of compound 1 in vivo against human breast cancer is shown in an
15 estrogen cancer initiated ACI rat breast cancer model. Approximately 90% of rats implanted with estrogen pellets develop breast cancer within 6 months after estrogen implantation.

Compound 1 is dissolved in 100% ethanol and is diluted to the appropriate dosage using vitamin E depleted peanut oil. The
20 maximum tolerated dose (MTD, maximum dose of compound that can be administered without adverse affects) is determined as described in

Examples 14 and 15. Compound 1 is administered at the maximum tolerated dose and 50% of the maximum tolerated dose. ACI rats at 4 weeks of age are subpannicularly implanted with estrogen pellets in the shoulder region. Compound 1 at maximum tolerated dose and 50% of the maximum tolerated dose is administered by gavage. Breast tumors are detected in the control group at approximately 100 days following estrogen implantation. Ninety percent of the control rats develop breast cancer within 6 months after estrogen implantation. Tumor bearing animals from control and treatment groups are sacrificed at various time intervals after treatment initiation, and mammary tissue is examined for obvious tumors, and further examined by histological analyses.

The following references are cited herein.

15

1. Colowisk, Sidney, P. and Kaplan, Nathan, O. *Methods in Enzymology*, Vol. XVIII, Vitamins and Coenzymes, Part C. Edited by Donald B. McCormick and Lemuel D. Wright. Section XII, PP. 335.
 2. Dhar, A., Liu, S., Klucik, J., Berlin, K.D., Madler, M.M., Lu, S., Ivey, R.T., Zacheis, D., Brwon, C.W., Nelson, E.C., Birchbichler, P.J., Benbrook, D.M. Synthesis, Structure-Activity Relationships, and RAR γ -Ligand
- 20

Interactions of Nitrogen Heteroarotinoids. *J. Med. Chem.* 42, pp. 3602-3614 (1999).

Any patents or publications mentioned in this specification
5 are indicative of the levels of those skilled in the art to which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was specifically and individually incorporated by reference.

10 One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently
15 representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.